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GENETICS OF A COLONIAL MICROCONIDIATING MUTANT STRAIN OF NEUROSPORA CRASSA*[1]

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WILD type strains of *Neurospora crassa* produce asexual spores in the form of multinucleate macroconidia and have a spreading growth habit. The value of this organism for biochemical and genetical research would be greatly increased if strains were available having restricted colony size and producing exclusively uninucleate microconidia. These characteristics would permit manipulation of Neurospora by bacteriological techniques and allow rapid establishment of homocaryotic lines. This paper reports the genetical analysis of a mutant strain (Y-8743) having these characteristics, the interaction of the genes concerned, and the development of derived strains.

Specifically, Y-8743 differs from the wild type in two clearly defined ways. First, it grows at a rate of less than 0.05 mm/hr, at 25°C, forming a button-like thallus, or colony, on semi-solid medium, and forming pellets in liquid shake cultures. Second, the only asexual spores produced are microconidia. In contrast, the wild type grows at about 4 mm/hr, produces a web-like mycelium on semi-solid medium, and forms a tangled mycelial mass in liquid shake culture. The asexual spores produced are predominantly multinucleate macroconidia.

EXPERIMENTAL

Materials and Methods

Strain Y-8743 was derived from an ascospore isolated from a cross involving material treated with sodium 20-methylcholanthrene-11,14-endo-α,β-succinate. Conidia of strain 1A were treated with this carcinogenic agent, added to protoperithecia of strain 25a, ascospores subsequently isolated, and the resulting cultures tested by the technique of BEADLE and TATUM (1941, 1945) for the detection of mutants. Originally strains 1A and 25a were isolated from a cross of LINDEGREN'S stocks La and LA (BEADLE and TATUM 1945). Other genes concerned with colonial growth, to be discussed later, occur in strains Y-5331 and Y-5296. The former originated from material treated with methyl-

* Part of the cost of the accompanying plate has been paid by the GALTON AND MENDEL MEMORIAL FUND.

[1] This work was supported by grants from the AMERICAN CANCER SOCIETY, recommended by the COMMITTEE ON GROWTH OF THE NATIONAL RESEARCH COUNCIL, and from the ROCKEFELLER FOUNDATION. A part of the data reported here is included in a dissertation submitted by the senior author to the Graduate School, YALE UNIVERSITY, in partial fulfillment of the requirement for the Degree of Doctor of Philosophy.

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TABLE 1

Genes used in the linkage studies

GENE CHARACTER	MUTANT NUMBER*	SYMBOL	LINKAGE GROUP		CENTROMERE DISTANCE**	NO. ASCI ISOLATED†
			NUMBER	REF.‡		
sex	—	*A* or *a*	I	(1)	7.8	755
albino-2	15300	*al-2*	I	(2)	27.4	919
peach	—	*pe*	II	(3)	11.2	381
fluffy	—	*fl*	II	(3)	29.0	632
leucineless	33757	*leu*	III	(4)	8.5	236
tryptophanless	10575	*tpt-1*	III	(4)	21.5*	242
pyridoxinless	44602 (37803)	*pdx* (*pdx*)	IV	(5)	10.1	360
pantothenicless	5531	*pan*	IV	(5)	26.3	332
cholineless	34486 (34542)	*chol-1* (*chol-1*)	IV	(5)	24.4	166
lysineless	33933	*lys*	V	(5)	3.5	99
isoleucine-valineless	16117	*i-val*	V	(5)	9.4	160
inositolless	37401	*inos*	V	(5)	26.3	249
riboflavinless	51602	*rib*	VI	(5)	2.0	123
adenineless	3254	*ad*	VI	(5)	1.8	248
sulfonamide-requiring	E-15172	*sfo*	VII	(5)	31.6	117

* Numbers with no letter preceding them identify the strain as originating at STANFORD UNIVERSITY, while the letter Y preceding a number identifies it as originating at YALE UNIVERSITY. The letter E refers to a strain obtained by DR. S. EMERSON at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

** Uncorrected for double crossovers.

† See table 1A for compilation of data.

‡ (1) C. C. LINDEGREN (1936), (2) M. V. G. HUNGATE (1945), (3) C. C. LINDEGREN and G. LINDEGREN (1939), (4) F. P. HUNGATE (1946). (5) Four additional linkage groups have been demonstrated making a total of seven which agrees with the cytological data (MCCLINTOCK 1945). These groups have been arbitrarily assigned numbers IV, V, VI, VII pending further standardization. Groups IV and V correspond to the 4th and 5th group from the left as figured by BEADLE (1946).

cholanthrene, the latter from material treated with nitrogen mustard. Table 1 lists the genes used in the genetical investigation, their symbols, their linkage groups, their centromere distances and the numbers of asci on which these values are based. Table 1A gives the compilation of data including the sources used to calculate centromere distances in table 1.

Crosses between strains were made on corn meal agar and on synthetic crossing medium (WESTERGAARD and MITCHELL 1947). The presence of a mutant gene for a biosynthetic reaction was determined by failure of a culture to grow on liquid minimal medium. In crosses where several genes controlling biochemical reactions were segregating, the requirements of a culture were determined by an omission series. The morphological characters were determined whenever possible on cultures grown directly from the ascospores on the standard Neurospora complete medium in 7.5×1.0 cm tubes. The presence of

TABLE 1A

Compilation of data used to calculate centromere distances in Table 1

NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*
TOTAL	2ND DIV. SEGREG.		
	sex		
449	58	6	11
178	36	10	12
16	2	6	1
50	7	7	4
62	14	11	authors
—	—	—	
755	117	7.8	
	albino-2 (15300)		
140	64	23	10
100	57	28	7
98	57	29	16
14	12	43	17
39	24	32	18
20	4	10	14
121	69	28	4
387	216	28	authors
—	—	—	
919	503	27.4	
	peach		
103	23	11	12
278	63	11	13
—	—	—	
381	86	11.2	
	fluffy		
110	68	31	11
278	157	27	13
27	13	24	9
217	128	29	authors
—	—	—	
632	366	29.0	
	isoleucine-valineless (16117)		
31	8	13	1
21	5	12	3
60	7	6	5
20	1	2	8
28	9	16	authors
—	—	—	
160	30	9.4	
	leucineless (33757)		
79	12	8	9
129	22	9	15
28	6	11	authors
—	—	—	
236	40	8.5	
	tryptophanless (10575)		
229	94	21	9
13	10	38	authors
—	—	—	
242	104	21.5	
	pyridoxineless (44602) (37803)		
332	70	11	2
28	3	5	authors
—	—	—	
360	73	10.1	
	pantothenicless (5531)		
257	135	26	2
75	40	27	authors
—	—	—	
332	175	26.3	
	cholineless (34486) (34542)		
139	70	25	2
27	11	20	authors
—	—	—	
166	81	24.4	
	lysineless (33933)		
71	4	3	6
28	3	5	authors
—	—	—	
99	7	3.5	
	adenineless (3254)		
10	1	5	9
99	4	2	8
37	3	4	5
20	0	<2	15
82	1	1	authors
—	—	—	
248	9	1.8	

TABLE 1A—*continued*

NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*	NUMBER OF ASCI		CENTROMERE DISTANCE	DATA SOURCE*
TOTAL	2ND DIV. SEGREG.			TOTAL	2ND DIV. SEGREG.		
inositolless (37401)				riboflavinless (51602)			
80	47	29	7	111	4	2	2
79	43	27	4	12	1	4	authors
90	41	23	authors	—	—	—	
—	—	—		123	5	2.0	
249	131	26.3					

* (1) E. A. ADELBERG (unpublished), (2) HOULAHAN, M. B., G. W. BEADLE, and H. G, CALHOUN (1949), (3) BONNER, TATUM and BEADLE (1943), (4) D. M. BONNER (unpublished). (5) H. R. BUSS (1944), (6) A. H. DOERMAN (1946), (7) N. H. GILES (unpublished), (8) M. B. HOULAHAN (1944), (9) F. P. HUNGATE (1946), (10) M. V. G. HUNGATE (1945), (11) C. C. LINDEGREN (1933), (12) C. C. LINDEGREN (1936), (13) C. C. LINDEGREN and G. LINDEGREN (1939), (14) D. E. NEWMEYER (unpublished), (15) D. C. REGNERY (1947), (16) A. M. SRB (1946), (17) H. J. TEAS (1947), (18) E. ZIMMER (1946).

the gene controlling microconidiation was determined by microscopic examination for microconidia or by segregation from outcrosses to suitable strains.

Centromere distances in map units were calculated by dividing by two the percentage of asci showing second division segregation (LINDEGREN 1933, 1936). The term serially (that is, orderly) isolated asci refers to those asci from which the ascospores were dissected and their order maintained. Unless stated otherwise, all isolations reported in this paper were serial.

Genes Controlling Mutant Characteristics in Strain Y-8743

In a preliminary experiment Y-8743a was outcrossed to the wild type (cross 3) and 100 ascospores were isolated at random. The results (table 2) give the following four classes in about equal numbers: (1) wild type (spreading-macroconidial), (2) pink clumpy, (3) colonial-sterile, and (4) colonial-microconidial as shown in figure 1. These frequencies show good agreement with the hypothesis that strain Y-8743 differs from wild type by two non-linked genes. Table 2 gives the results of the Chi Square test on these data and lists the symbols adopted for each class; P equals 0.78, which is satisfactory agreement between observed and expected. Since in cross 3 only 77 spores germinated out of 100 isolated, the existence of a fifth and lethal class could not be precluded. To explore this possibility, Y-8743a was crossed with 1A (cross 1) and spores of nine asci isolated serially. In six asci the same four classes were recovered while in three asci only two types segregated. An additional intercross between *col-1, M* and *Col-1, m* (cross 8) also gave rise to no new types in 13 asci. Since only four classes were obtained in the progeny from all the crosses, it was concluded that strain Y-8743 differs from the wild type by two genes.

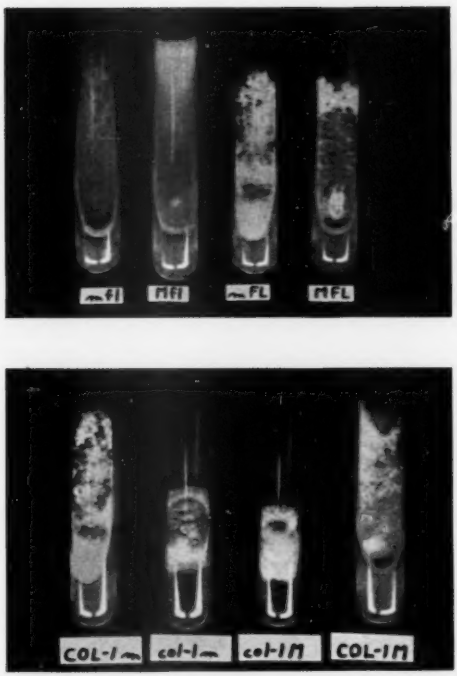

FIGURE 1.—Ascus segregations of *Neurospora crassa*. Upper: segregation types from cross of fluffy with pink-clumpy showing first division segregation of fluffy and second division segregation for microconidial. Left to right, fluffy microconidial (*m*, *fl*), fluffy (*M*, *fl*), pink-clumpy (*m*, *Fl*) and wild type (*M*, *Fl*). Lower: segregation of Y-8743 showing first division segregation of microconidial and second division segregation of colonial-1. Left to right, pink-clumpy (*Col-1*, *m*), colonial microconidial (*col-1*, *m*), colonial sterile (*col-1*, *M*), and wild type (*Col-1*, *M*).

Some interaction of genes was apparent because one of the classes, colonial-sterile (*col-1, M*) although it contains the allele *M*, produces no asexual spores, and another class, pink clumpy (*Col-1, m*) although it contains the allele *m*, produces both macro- and microconidia. In addition to producing both macro- and microconidia, a strain of the genotype *Col-1, m* shows a somewhat different growth habit and coloration from the wild type (see fig. 1). The spores, mostly macroconidia, are borne all over the surface of an agar medium rather than predominantly at the top of a slant or at the outer margin of a Petri plate as in wild type cultures. This distribution of conidia gives the culture a clumpy appearance. Several genes modifying this clumpy character have been noted but have not been investigated. Cultures of the genotype *Col-1, m* are pink

TABLE 2

Chi Square test on random isolates from outcross of Y-8743 (cross 8743-3)

MORPHOLOGICAL CHARACTERIZATION		SYMBOL	OBSERVED FREQUENCY
TYPE OF GROWTH	CONIDIATION		
spreading	macroconidia	*Col-1, M*	20
spreading	macroconidia+microconidia	*Col-1, m*	23
colonial	none	*col-1, M*	15
colonial	microconidia	*col-1, m*	19
		total	77

$\chi^2=1.698$; P=0.78 (on hypothesis that the mutant differs from the wild type by two independently segregating genes).

or peach rather than orange as in wild type. The pink character is always expressed in cultures differing from the wild type by the gene *m*. For these reasons the genotype *Col-1, m* has been called pink clumpy. The genetics of genes colonial-1 (*col-1*) and microconidial (*m*) will be discussed separately.

Gene controlling colonial growth (colonial-1)

To determine whether or not a single gene differentiates the colonial character from the wild type, the usual crosses were made. A colonial-sterile (*col-1, M*) isolate was outcrossed to the wild type (*Col-1, M*) (cross 10) and spores of 21 asci were isolated. Cultures grown from ascospores showed four *col-1, M* and four *Col-1, M* genotypes in each ascus. An intercross (cross 12) between *col-1, m* and *Col-1, m* was made and the spores of 18 asci dissected. Again only the two parental types were recovered. An additional cross was made between two colonials of the genotypes *col-1, m* and *col-1, M*. This colonial by colonial cross produced poor perithecia and all the spores of only two asci germinated. All spores, including those from the complete and incomplete asci, gave rise to cultures of colonial growth type. The above data show that the single gene, colonial-1 (*col-1*), differentiates between a culture derived from Y-8743 having colonial growth and one having spreading growth.

TABLE 3

Centromere data on Col-1 locus

CROSS NUMBER	NUMBER OF ASCI: 2ND DIVISION SEGREGATION	NUMBER OF ASCI: TOTAL	CENTROMERE DISTANCE	*col-1* PARENT	*Col-1* PARENT
8743-1	5	9	27.8	Y-8743a	1A
8743-8	8	15	26.8	Y-8743-3-2	Y-8743-3-69
8743-10	14	21	33.3	Y-8743-3-17	Y-8743-3-69
8743-12	11	18	30.5	Y-8743-3-3a	Y-8743-3-55A
8743-22	10	17	29.4	Y-8743-2-6A	multiple
8743-25	13	29	22.4	Y-8743-2-6A	44602-1-2a
8743-26	9	17	26.5	Y-8743a	SY-7A
8743-28	4	10	20.0	Y-8743-2-6A	Y-8743-21(1-1)a
8743-29	13	27	24.1	Y-8743-2-6A	34486a
8743-45	5	9	27.8	Y-8743-2-6A	Y-8743-1(8-3)a
SY-8743-1	1	4	12.5	Y-8743-2-6A	SY-4a-f8
Total and average	93	176	26.4		

$\chi^2 = 5.28$; n = 10; P = 0.88.

Table 3 shows the segregation of the *col-1* allele in 176 asci from 11 crosses; *col-1* is located 26.4 map units from the centromere as calculated from data uncorrected for double crossing over. A Chi Square test for homogeneity of the data in the 11 crosses gives a value of 5.28. P calculated from this equals 0.88, which substantiates the data as homogeneous.

MCCLINTOCK (1945) has shown *Neurospora crassa* to have seven chromosomes. GILES (unpublished) has combined characters from five linkage groups (I *al-2* and *sex*, III *tpt-1*, IV *pan*, V *inos*, VI *rib*) into one genotype. In order to determine whether or not gene colonial-1 is in any of these groups, Y-8743 was crossed with this multiple stock (cross 22) and spores of 14 asci isolated. The recombinations of the *col-1* gene with the markers are given in table 4.

TABLE 4

Recombination of genes in "multiple stock" (see text) with col-1

LINKAGE GROUP	GENE	NO. ASCOSPORE PAIRS: PARENTAL	NO. ASCOSPORE PAIRS: RECOMBINATION	% RECOMBINATION
I	*al-2*	23	23	50.0
III	*tpt-1*	26	20	43.5
IV	*pan*	46	0	00.0
V	*inos*	26	20	43.5
VI	*rib*	30	16	35.0

Since the gene *col-1* shows no recombination in 46 ascospore pairs with *pan* and yet shows essentially random recombination with the other four markers, it was placed tentatively in linkage group IV. Crosses were made with cultures having genes known to be in the same arm of linkage group IV as follows: *pdx*, located 10.1 map units from centromere (cross 25); *chol-1*, located 24.4 map units from centromere; and additional data were obtained on linkage with *pan* which is 26.3 units from the centromere (cross 49). No recombinations were observed between *col-1* and *pan* in 194 ascospore pairs (table 5). This obvi-

TABLE 5

Linkage of Col-1 with genes in linkage group IV
(centromere distance Col-1=26.4 units)

GENE CHARACTER MUTANT NUMBER	CENTROMERE DISTANCE*	NO. ASCOSPORE PAIRS			% CROSSINGOVER		CHI SQUARE‡	P
		ISOLATED	OBSERVED RECOMBINED	EXPECTED RECOMBINED**	OBSERVED	EXPECTED**		
Pyridoxineless 44602	10.1	112	24	18.2	21.4	16.3	0.92	0.35
Cholineless 34486	24.4	108	8	2.2	7.4	2.0	7.6	<0.01
Pantothenicless† 5531	26.3	46	0	0.05	0.0	0.1	0.01	
		148	0	0.15	0.0	0.1	0.03	
		194	0	0.20	0.0	0.1	0.04	0.97

* Not corrected for double crossovers.
** Calculated from centromere values.
† Data from two crosses (n=2).
‡ Calculated from chromatid pairs (number ascospore pairs divided by 2).

ously shows good agreement with the expected crossing over based on their centromere distances. The linkage data between *col-1* and *chol-1*, and between *col-1* and *pdx* show poor agreement between the observed and expected crossing over based on centromere data. The *col-1*×*pdx* cross gave 21.4 percent of crossing over while only 16.3 percent was expected based on centromere distances. The *col-1*×*chol-1* cross gave 7.4 percent of crossing over while only 2.0 percent was expected based on the centromere distances. Since the centromere distances are uncorrected for double crossovers, the linkage data no doubt give a more valid estimate of the true gene relationships than that obtained by comparing centromere distances.

It was concluded from these results that the *col-1* locus is in linkage IV, very closely linked (within one unit) with *pan* and about 7.4 units from *chol-1* and 21.4 units from *pdx*.

Gene controlling microconidiation (m)

To determine whether or not a single gene controls microconidiation in strain Y-8743, crosses were made between *Col-1, m* and each of the following biochemical mutants having wild type morphology: *ad* (cross 3254-1), *i-val* (cross 39), and *leu* (cross 36). Altogether, the spores from 76 asci were analyzed. Four pink clumpy (*Col-1, m*) and four wild type cultures (*Col-1, M*) were obtained from each ascus and no other morphological type appeared. In two asci dissected from the cross *col-1, m* by *col-1, M* (previously mentioned) only parental types were recovered. Accordingly, microconidiation is controlled by the single gene *m* in strain Y-8743.

TABLE 6

Centromere data on M locus

CROSS NUMBER	NUMBER OF ASCI		CENTROMERE DISTANCE	*m* PARENT	*M* PARENT
	2ND DIVISION SEGREGATION	TOTAL			
8743-1	2	8	12.5	Y-8743a	1A
8743-11	1	22	25.0	Y-8743-3-53A	Y-8743-3-59a
8743-13	3	7	21.5	Y-8743-3-39A	37401a
8743-14	3	13	11.5	Y-8743-3-3a	Y-5331A
8743-15	4	22	9.1	Y-12504-1-13A	37401a
8743-21	9	18	25.0	Y-8743-14(11-1)a	Fluffy A
8743-22	3	14	10.7	Y-8743-2-6A	multiple a
8743-24	2	14	7.2	Y-8743-13(19-5)a	16117A
8743-25	5	28	8.9	Y-8743-2-6A	44602-1-2a
8743-26	5	13	19.2	Y-8743a	SY-7A
8743-27	1	12	4.2	Y-8743-21(5-3)A	SY-4a
8743-28	3	10	15.0	Y-8743-2-6A	Y-8743-21(1-1)a
8743-29	7	26	13.5	Y-8743-2-6A	34486a
8743-30	4	10	20.0	Y-8743-21(2-1)	Y-8743-24(13-5)
8743-31	2	6	16.7	Y-8743-21(13-7)a	4540A
8743-32	3	9	16.7	Y-8743-21(5-3)A	3416a
8743-34	5	11	22.7	Y-8743-21(13-7)a	4545A
8743-35	11	35	15.7	Y-8743-3-3a	E-15172(2-35-I-9)
8743-36	3	29	5.2	Y-8743-3-39A	33757a
8743-37	5	10	25.0	Y-8743-21(13-7)a	5531A
8743-39	7	22	15.9	Y-8743-3-39A	16117a
8743-40	7	21	16.6	Y-8743-21(5-3)A	33933a
8743-41	2	8	12.5	Y-8743-1(9-1)a	SY-7A
8743-42	1	5	10.0	Y-8743-1(9-2)a	SY-7A
8743-43	1	6	8.3	Y-8743-1(8-3)A	SY-4a
3254-1	14	33	21.2	Y-8743-3-3a	3254-6381-5A
Total and average	113	392	14.4		

$\chi^2=26.3$; n=25; P=0.40.

Table 6 shows the segregation of *m* in 392 asci from 26 crosses. The gene *m* is located 14.4 units from the centromere as calculated from data uncorrected for double crossing over. A Chi Square test for homogeneity of the data from 26 crosses gives a value of 26.3. P calculated from this equals 0.40.

Gene *m* recombines at random with the five markers in the multiple stock previously described (cross 22) (see table 7), hence does not appear to be located in any of these groups. Wherever possible, these linkage groups were further checked by making additional crosses with strains marked in both

TABLE 7

Recombination of genes in multiple stock (see text) with gene m

LINKAGE GROUP	GENE	NO. ASCOSPORE PAIRS		% RECOMBINATION
		PARENTAL	RECOMBINATION	
I	*al-2*	18	30	62.5
III	*tpt-1*	26	22	45.8
IV	*pan*	32	16	33.3
IV	*col-1*	32	20	39.3
V	*inos*	24	24	50.0
VI	*rib*	34	18	34.6

arms at centromere distances corresponding to that of *m*. The data in table 8 show that the gene *m* is not in linkage groups I, III, IV, V, or VI. Other crosses were made with peach (*pe*) and fluffy (*fl*) in linkage group II; and with *sfo*, the sulfonamide-requiring strain E-15172 (Emerson 1947) which is not in groups I, III, IV, V, VI (Emerson, personal communication) nor in II according to our data, and therefore is assumed to be the first marker in group VII. Gene *m* showed no recombination in 200 ascospore pairs with *pe* and 31.5 percent recombination with *fl*, while it showed random assortment with *sfo* (table 8). Therefore *m* is located in linkage group II,[3] and is closely linked to or identical with peach. Cultures of strains containing the gene *pe* appear the same as cultures containing the gene *m*, thus substantiating their identity. Furthermore, a heterocaryon between *pe*, *ad* and *pan*, *m* on minimal agar results in a culture having the typical morphology of *pe* or *m*. Recalculation of the LINDEGRENS' (1939) recombination data on *pe*, *tu*, and *fl* gives 24.9 percent crossing over between *m* and *fl* (140 asci) (table 8). The centromere values of *m* and *pe*—14.4 and 11.3—show about the same percentage difference (27 percent) as the crossover measurements between *m* and *fl*, and *pe* and *fl*—31.5 to 24.6—or 28 percent. The constancy of the percentage differences between our data and the Lindegrens' suggests that the two sets were collected under different environments such that chiasma frequency was affected. From the above data and reasoning it is concluded that the gene designated *m* throughout this paper is the same locus as *pe*. Since microconidiating stocks designated as *m* were in

[3] Recently in this laboratory a biochemically deficient strain (Y-2492) has been found to be closely linked to fluffy.

TABLE 8

Recombination data of gene m with marker genes in each linkage group (centromere distance gene m = 14.4)

GENE CHARACTER		CENTROMERE DISTANCE MAP UNITS	NO. ASCOSPORE PAIRS		PERCENT RECOMBINATION		
LINKAGE GROUP AND ARM	MUTANT NUMBER		ISOLATED	RECOMBINED	OBSERVED	EXPECTED* RIGHT ARM	EXPECTED* LEFT ARM
I Right	Albino-2 15300	27.4	542	294	54.4	13.0	—
Left	Sex	7.8	129	69	53.5	—	6.6
II Right	Peach (LINDEGREN)	11.2	200	0	0.0	3.2	—
	Fluffy (LINDEGREN)	29.0	559	176	31.5	14.6	—
III Right	Leucineless 33757	8.5	118	70	59	5.9	22.9†
	Tryptophaneless 10575	21.5	48	22	45.9	7.1	—
IV Right	Pyridoxineless 44602	10.1	112	58	51.8	4.3	24.5†
	Cholineless 34486	24.4	108	50	46.3	10.0	—
	Colonial-1 Y-8743	26.4	404	197	48.7	12.0	—
	Pantothenicless 5531	26.3	168	78	46.5	11.9	—
V Right	Isol.-valineless 16117	9.4	132	56	42.4	5.0	—
	Inositolless 37401	26.3	180	80	44.4	11.9	—
Left	Lysineless 33933	3.5	114	64	56.2	17.9†	10.9
VI Right	Adenineless 3254	1.8	66	31	46.9	12.6	16.2†
	Riboflavinless 51602	2.0	52	18	34.6	12.4	16.4†
VII Right	Sulfonamide-requiring E-15172	31.6	136	64	47.1	17.2	—

* On basis of linkage assuming gene *m* on right or left arm as indicated.

† Checked across centromere. These data are much less reliable than indicated because the errors of double crossovers are additive when genes are on opposite sides of centromere.

use in several laboratories prior to this conclusion and the genetics of strain Y-8743 was presented at the Eighth International Congress of Genetics, the authors suggest that this allele be called *pe*^{m} to alleviate future confusion.

Dominance and reversion

The term dominance was first used to describe the condition in a diploid organism heterozygous for a character in which one allele is expressed to the exclusion of the other. Dominance is a measure of allele activity at an arbitrary 1:1 ratio. It gives no quantitative evaluation of the activity such as would be given by the minimum allele ratio at which one allele is expressed to the exclusion of the other. PONTECORVO (1946) has recently discussed in detail the similarities and differences between the diploid condition and that existing in a heterocaryon (dicaryon) in a haploid organism. BEADLE and COONRADT (1944) have shown that in Neurospora the use of heterocaryons provide a general method for studying dominance relations between mutant genes and their normal alleles; and that in a heterocaryon between two mutant strains the nuclear ratio can vary over wide ranges depending upon the component nuclei. They found certain alleles—especially pantothenicless—which led to wild type growth in a heterocaryon when in allele ratios as one-sided as 15:1 and 17:1. In an extension of this approach SANSOME (1947) has pointed out that heterocaryons offer an important new field for the study of gene expression as related to allele dosage.

In the present investigation an experiment using heterocaryons was conducted to determine the dominance relations between *col-1* and *Col-1*, that is, the effect at a 1:1 allele ratio. Mixtures of macro- and microconidia were made in varied ratios (table 9) and inoculated into flasks of liquid complete medium. The microconidia were from a strain of genotype *col-1, m*, pregerminated for 24 hours in a complete medium at 25°C. The macroconidia were taken directly from a 48 hour agar slant culture of a strain of genotype *Col-1, m*. The flasks were shaken for 72 hours at 25°C in order to provide the opportunity for heterocaryon formation. "Growth tubes" containing a complete agar medium

TABLE 9

Growth rates of heterocaryons between col-1, M and Col-1, m in various nuclear ratios

NO. CONIDIA PER FLASK		RATIO $\frac{\text{MICRO}}{\text{MACRO}}$	PROBABLE NUCLEAR RATIO	GROWTH RATE MM/HR
MICRO (*col-1*)	MACRO (*Col-1*)			
5×10^{5}	0	∞ :0	∞ :0	0.01
5×10^{5}	5×10^{2}	1000:1	100:1	0.55
5×10^{5}	5×10^{3}	100:1	10:1	1.12
5×10^{5}	5×10^{4}	10:1	1:1	2.95
2.5×10^{5}	2.5×10^{5}	1:1	1:10	3.00
5×10^{4}	5×10^{5}	1:10	1:100	3.00
0	5×10^{5}	0: ∞	0: ∞	3.00

were inoculated with mycelium from the flasks and incubated at 25°C. Inoculation of the growth tubes from the flasks was found to be a necessary step since direct inoculation of the growth tubes by mixtures of macro- and microconidia resulted in the wild type overgrowing the colonial strain regardless of the ratios. The tubes were marked twice daily and the growth rates of the heterocaryons calculated by plotting time in hours against distance in millimeters. Heterocaryons made at each ratio showed straight line plots indicating no selection of either nuclear type during growth along the growth tube. Although no check was made for the actual formation of heterocaryons and no biochemical markers were used to assure heterocaryosis, nor was the nuclear ratio determined at the end of the growth tube; nevertheless, the quantitative response shown in table 9 and the straight line growth curves are considered reasonable evidence for the formation of heterocaryons. The best available estimate of the nuclear ratio is given in column four of table 9 which is calculated on the assumption that a macroconidium contains about ten nuclei (1–20 or more have been observed). It seems reasonable to conclude that at a nuclear ratio of approximately 1:1 with respect to alleles *col-1* and *Col-1*, the wild type allele is dominant.

The dominance relations between gene *m* and its wild type allele *M* have not been investigated.

The *col-1* gene reverts spontaneously to an allele mediating much more rapid growth rate. LINDEGREN and LINDEGREN (1941b) also reported the spontaneous reversion of their colonial strain "button" to the wild type. Results obtained from plating microconidia from a 10 day old culture of genotype *col-1*, *m* show a reversion frequency of the order of magnitude of between 1 in 10^6 and 1 in 10^7 nuclei. Whether or not the reversions are to alleles other than the wild type has not yet been determined. The possibility that the reversion is due to a mutation at another locus giving rise to a suppressor of colonial growth has not been eliminated.

The gene *m* has never been observed to revert to the allele *M* but exhaustive tests have not been made to examine this point.

Other genes affecting colonial growth

Strains having a colonial growth habit have been recorded in the literature several times. LINDEGREN and LINDEGREN (1941b) describe a mutant obtained from ultraviolet treated material called "button" which, they state, produces colonies six mm in diameter. The Lindegrens' data indicate that button differs from the wild type by a single gene. BEADLE and COONRADT (1944) used two colonial mutants (2608 and 3100) and two semi-colonials (221 and 5801) in their heterocaryon studies. They defined a culture as colonial when the growth rate was less than 1.0 percent of normal, and as semi-colonial, when the rate was about 1.0 percent of normal. BONNER (1946), HOROWITZ, HOULAHAN, HUNGATE, and WRIGHT (1946), MCELROY, CUSHING and MILLER (1947), and MILLER and MCELROY (1948) all reported colonial strains derived from material treated with mustard gas and nitrogen mustard.

In this investigation experiments have been made with two other colonial

growing strains (Y-5331 and Y-5296). A heterocaryon between these two strains grows at a wild type rate (Dr. KATHERINE WILSON, personal communication), which shows that the two strains differ genetically. Dr. WILSON has made hyphal tip isolates from the heterocaryon and has demonstrated the presence of each type of nucleus. Colonial-1 and Y-5331 also form a heterocaryon which has wild type growth rate. To date it has been impossible to form a heterocaryon between *col-1* and Y-5331 showing a wild type growth rate. These tests plus data to be presented in the following section prove the three strains to be genetically different. Y-5331 has been termed colonial-2 (*col-2*) and Y-5296, colonial-3 (*col-3*).

Ascospores from 13 asci were isolated from the cross of Y-5331 by a strain having the genotype *Col-2*, *m* (cross 14-2). All asci segregated with four colonial and four spreading growth cultures showing that Y-5331 differs from the wild type by the single gene *col-2*. A similar cross between Y-5296 and *Col-3*, *m* (cross 14-1) was made and the ascospores from six asci were isolated. These asci also showed a typical Mendelian segregation for a single gene difference. Thus Y-5331 and Y-5296 each represent a single gene difference from the wild type. Spontaneous reversion to strains having a more rapid growth rate has been observed in *col-2* cultures. Ascus segregation data suggest that genes *col-2* and *col-3* are both within ten map units of the centromere.

Interaction of genes

It has been shown that the gene *m* in a wild type genotype is expressed as a pink clumpy culture forming both macro- and microconidia but forming predominantly macroconidia. In the presence of *col-1*, the gene *m* results in the production of microconidia only. In the presence of the *M* allele, a culture with the genotype *col-1* is conidialess. Therefore the gene *col-1* affects the phenotypic expression of the alleles *m* and *M*.

Y-5331, differing from the wild type by the single gene *col-2*, is a strain producing neither macro- nor microconidia. Cross 14-2 between Y-5331 (*col-2*) and a pink clumpy stock (*Col-2*, *m*) was made and the spores of 13 asci dissected. The ascus isolations show two genes segregating—*col-2* and *m*—demonstrating that Y-5331 contains the wild type allele *M*. The genotype *col-2*, *m* produces microconidia abundantly. Thus the situation is essentially the same as with *col-1;* in the presence of *m* both colonial genotypes produce microconidia whereas in the presence of *M* both are conidialess.

Y-5296, differing from wild type by the single gene *col-3*, is also a colonial strain producing neither macro- nor microconidia. Cross 14-1 between Y-5296 and a strain known to differ from wild type by the gene *m* was made and the spores from 11 asci isolated. The germination of the ascospores from this cross was poor with all the segregation types represented in only five asci. In these asci no conidiating colonial types were obtained although in several cases the ascus segregation was such that the gene *m* was present with *col-3*. Thus the situation is different with *col-3* than with either *col-1* or *col-2;* since in the presence of *col-3* and either allele *m* or *M*, no conidia are produced.

In strains differing from wild type by the gene albino-2, a small amount of

pinkish pigment is produced in the conidia, especially in cultures exposed to continuous illumination. Miss PATRICIA ST. LAWRENCE (personal communication) has observed that this pigmentation is increased by using glycerol instead of sucrose as a carbon source. She has also observed that no pigment whatever is developed in albino cultures having the gene *m* regardless of the type of media used to date. Thus the gene *m* changes the pigment from orange to pink or peach in otherwise wild type cultures and completely prevents any pigment formation in albino-2.

Microconidia in Neurospora

Microconidiation was first described in *N. sitophila* by DODGE (1930). In a later paper (1932) he reported microconidiation in *N. tetrasperma* and *N. crassa* and found the character to be inherited. He believed that microconidia (microspores or spermatia), although produced by both sexes, represent ancestral male elements which can revert to perform a vegetative function.

DODGE (1930) described microconidia as "minute hyaline spherical to pear-shaped bodies about 2.5 to 3.5 microns in diameter" and observed that they can germinate directly into a hypha, but more slowly than macroconidia. Our measurements of 45 microconidia gave an average size of 2.43×3.98 microns (2.0×3.0 to 3.0×5.0). Our data substantiate the observation that microconidia germinate more slowly than macroconidia. Colonies appear in 36 hours at 35°C from microconidia of spreading strains as contrasted with less than 18 hours at 35°C from macroconidia. Ninety-five percent of the colonies appear within 62 hours at 25°C on a complete medium from microconidia of colonial microconidiating strains.

It is well known that ordinary monilioid conidia, or macroconidia, contain several nuclei (see fig. 2). The reported evidence concerning the nuclear condition of microconidia is somewhat incomplete. DODGE (1932) concluded that "because of their very small size, they (microconidia) are probably, in general, uninucleate." ZICKLER (1937) examined fixed and stained microconidia of *Bombardia lunata*, a closely related genus, and found them to be uninucleate. LINDEGREN and LINDEGREN (1941a, 1941b) and SANSOME, DEMEREC and HOLLAENDER (1945) stated that *N. crassa* produces uninucleate microconidia but offered no evidence.

Inadequate cytological techniques in the past have prevented use of the *direct* method in determining the number of nuclei in Neurospora microconidia but recently well fixed and clearly differentiated preparations have been obtained (fig. 2) with ROBINOW's techniques (1942, 1944, and personal communication) using acid Giemsa stain.[4] Examination of several thousand microconidia at various ages showed them to be more than 99 percent uninucleate. The number of nuclei in macroconidia, using the same technique, varies from one to 20 or more.

Also an *indirect* method was devised which gives supporting evidence of the

[4] Dr. V. M. CUTTER has also obtained clearly differentiated preparations with the technique of DELAMATER (1948).

uninucleate nature of microconidia. A heterocaryon was established by inoculating the two deficient strains—*col-1, m, pdx* and *col-1, m, n-t*[5] onto a minimal agar slant. Microconidia produced from this heterocaryon were plated on a complete medium and 151 colonies isolated. Transfers from these colonies were tested on (1) minimal agar, (2) minimal agar plus nicotinamide (2 micrograms/cc), and (3) minimal agar plus pyridoxine (1 microgram/cc). No colo-

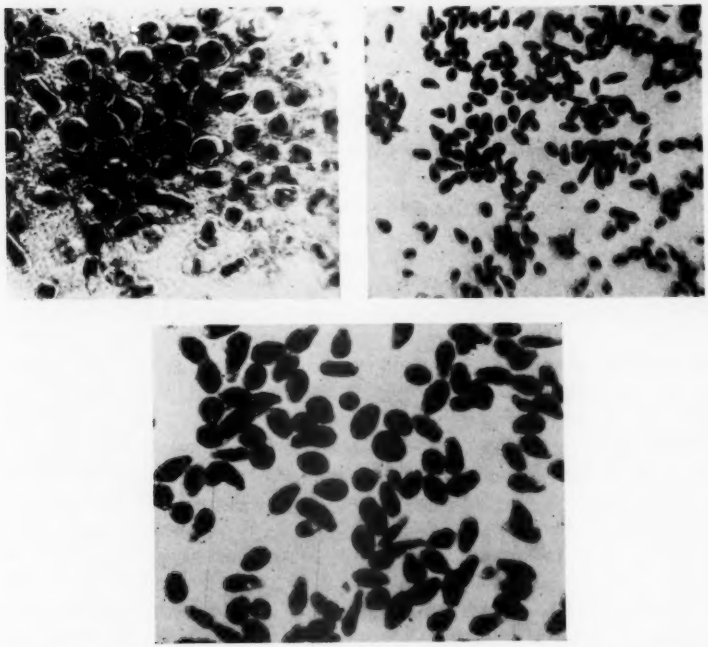

FIGURE 2.—Conidia of *Neurospora crassa*. ROBINOW's acid Giemsa stain. Upper right: microconidia of strain Y-8743-21(13-7)a showing one nucleus in each conidium. ×1375. Lower: the same, ×2850. Upper left: macroconidia of wild type strain SY 7A showing several nuclei in each conidium. ×1375.

nies grew on the minimal medium (1 above); all grew on either the nicotinamide or pyridoxine supplemented medium (2 or 3); and none grew on both the supplemented media (2 and 3). Therefore, microconidia produced from the genotype *col-1, m* are homocaryotic, and probably uninucleate. The experiment was repeated with two strains of the genotype *Col-1, m, fl*, each marked with a biochemical character. Microconidia from the heterocaryon of *tpt-1* and *lys* were plated directly on (1) a minimal medium, (2) a minimal medium plus

[5] *n-t* is the symbol for nicotinic-tryptophaneless (39401) (Beadle, Mitchell, & Nyc, 1947). The strain used was Y-17000 shown by D. M. BONNER to be the same as 39401 (personal communication).

lysine, and (3) a minimal medium plus tryptophane. Tergitol #7 was added to the media to produce discrete colonies (details to be reported elsewhere). At spore concentrations giving 636 colonies per plate on lysine and 288 colonies per plate on tryptophane, no colonies appeared on the plates containing only the minimal medium. SANSOME very recently (1947) showed somatic segregation from a heterocaryon of two component mutant types each having the gene fluffy by plating microconidia. She concluded that microconidia are "normally uninucleate." This experimental approach can be criticized on the basis that a homocaryotic microconidium may not necessarily be uninucleate. PONTECORVO (1946) stressed the fact that all the nuclei in a macroconidium are of the same genetic type in certain species of fungi known to have multinucleate conidia. Thus all that platings of microconidia from a heterocaryon can prove is that the microconidia are homocaryotic.

LINDEGREN (1933) described a macroconidialess strain, fluffy, which originated as a spontaneous mutation in selfed normal lines. He showed that it differed from wild type by a single gene located in linkage group II (LINDEGREN and LINDEGREN 1939). Fluffy normally produces no macroconidia or microconidia, but when mycelium is moistened and allowed to stand 24 hours, microconidia develop. These facts suggested that a combination of the macroconidialess character fluffy with the gene *m* might produce a new abundantly microconidiating strain with spreading growth habit. A cross of the genotype *col-2*, *m*, *Fl* (*col-1*, *m*, *Fl* can be substituted, with the same results) was made with fluffy, *Col-2*, *M*, *fl* (cross 21), and 20 asci dissected serially. In addition to the parental types, two others appeared having the genotype *col-2*, *M*, *Fl* and *Col-2*, *m*, *fl* as shown in figure 1. *col-2*, *M*, *Fl* is the same as Y-5331 as expected. *Col-2*, *m*, *fl* on the other hand, grows in a wild type spreading growth habit since it has the wild type allele *Col-2*, produces no macroconidia since it contains the *fl* gene, and produces microconidia abundantly since it contains the gene *m*.

A striking difference in time of conidiation exists between the microconidiating colonial strains and the microconidiating spreading strains. Y-8743 and Y-8743-2-6A produce microconidia in about ten days after transfer whereas Y-8743-21(13-7)a—a microconidiating spreading strain—produces microconidia on the fourth day. Spreading strains conidiate most abundantly when glycerol is substituted for sucrose as a carbon source in the complete agar medium. The reverse is true for colonial strains. Both types conidiate best at 25°C.

DISCUSSION

As described above, a strain containing one or more of the mutant genes *col-1*, *col-2*, and *col-3* is conidialess. Thus gene *M* controlling macroconidiation is hypostatic to all three colonial growth-controlling genes. In genotypes containing the gene *m* in addition to *col-1* or *col-2*, microconidia are produced abundantly. *m* is epistatic to or independent of *col-1* and *col-2*. In genotypes containing *col-3* and the allele *m* no microconidia are produced. Thus allele *m* as well as *M* is hypostatic to colonial-3. A plausible interpretation of these relationships is that the action of the colonial genes result in the loss of the

capacity to synthesize some unknown intermediate necessary for the production of macroconidia, the colonial character being a secondary expression of this loss. If this were the case, then in the presence of *col-1* or *col-2*, gene *m* would mediate the production of microconidia. In the presence of the wild type allele of these colonial genes, the action of the gene *m* is suppressed and a pink clumpy culture is obtained. Perhaps this suppression is the result of competition for an intermediate used in the production of macroconidia. Loss of function at the *col-3* locus leads to a block in conidiation prior to an intermediate necessary for the production of *any* conidia. Loss of function at the *fl* locus interrupts the production of macroconidiation without the secondary effect which results in colonial growth. In the presence of both gene *m* and gene *fl*, microconidia are produced abundantly.

Two origins of the alleles *m* and *col-1* should be considered. Either they are spontaneous mutations existing in the stock prior to treatment or were induced by the treatment. At the time when strain Y-8743 was isolated, phenotypically pink clumpy strains frequently (up to eight percent) appeared in isolations from control crosses of strain 1A by 25a. This suggests that the allele *m* occurred as a spontaneous mutation in the 1A or 25a stock and was present in the nucleus in which *Col-1* mutated to *col-1*. Colonial strains have never appeared in isolates from control crosses in this laboratory. Thus the mutation to colonial-1 probably occurred at the time of treatment with the carcinogen.

Strains of Neurospora containing the genes *m* and *col-1*, or *m* and *fl*, have promise as research tools. The colonial character permits direct plating whereas the uninucleate microconidial character allows the rapid establishment of homocaryotic lines. Data to be published elsewhere indicate that microconidia can be treated with mutagens or candidate mutagens and plated directly. The resulting homocaryotic cultures can be tested directly for biochemical mutants. These methods greatly facilitate the screening of chemicals as mutagens and the quantitative evaluation of known mutagens.

Genes controlling biochemical requirements have been introduced into genotypes containing *col-1* and *m*. GILES and E. LEDERBERG (1948) have used these colonial microconidiating strains in studying reversion rates of genes controlling biochemical requirements.

Strains having a wild type growth rate and producing only uninucleate microconidia have been developed by replacing the *col-1* gene with the gene *fl*. These microconidiating strains show promise in determining nuclear ratios in heterocaryons as begun by BEADLE and COONRADT (1944), in studying reversion, and in investigating mutagens.

SUMMARY

1. Strain Y-8743 differs from the wild type by two non-linked genes called colonial-1 (*col-1*) and microconidial (*m*).

2. The gene colonial-1 controls colonial growth and in the absence of *m* results in a conidialess mycelium. Colonial-1 is located in linkage group IV, 26.4 map units from the centromere as calculated from ascus segregation data uncorrected for double crossovers.

3. Gene *m* controls the production of microconidia in the presence of *col-1*. In the presence of the wild type allele (*Col-1*), the result is a pink clumpy culture producing both macro- and microconidia. Gene *m* is located in linkage group II 14.4 units from the centromere as calculated from ascus segregation data uncorrected for double crossovers. Gene *m* either is identical with or an allele of LINDEGREN's gene peach. It is suggested that the allele be designated by the symbol pe^{m}.

4. Two other independently occurring colonial mutant strains of *Neurospora crassa* were investigated. Colonial growth in both of these strains (designated colonial-2 and colonial-3) is the result of a single gene difference from the wild type, and both differ genetically from colonial-1.

5. Examination of fixed and stained microconidia at various ages showed them to be more than 99 percent uninucleate. Microconidial analysis of heterocaryons demonstrated microconidia to be greater than 99 per cent homocaryotic.

6. When the gene fluffy, whose action prevents macroconidiation, is genetically combined with the microconidial gene, a strain results which produces microconidia exclusively and has a wild type growth rate. Genotypes of Neurospora containing the genes *m*, *col-1*, and *fl* in various combinations are useful as research material.

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SELECTIVE MATING IN DROSOPHILA MELANOGASTER[1]

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ALTHOUGH DARWIN (1871) believed that sexual selection was quite distinct from natural selection, the modern definitions of natural selection (HALDANE 1932; HUXLEY 1938, 1942) include the mating process among the forces of natural selection. DARWIN's emphasis on the importance of male competition and the preferences of the females in sexual selection has been shown to have been misplaced in most cases. However, it is realized that the true measure of success by the individual, in an evolutionary sense, is not merely survival, but the number of offspring produced. Therefore, any understanding of the nature of the genetic changes occurring within a population requires some knowledge of the sexual behavior and the mating system.

Since such diverse factors as mutation pressure, migration, and isolation, as well as selection, can cause evolutionary change, a measuring device applicable to all was desirable. The conditions under which these and other factors are most effective in causing genetic changes, and the results of each acting separately or in combination have been predicted theoretically by the statistical treatment of gene frequencies in populations (FISHER 1930; HALDANE 1932; WRIGHT 1931, 1940a). The calculations have been greatly simplified by the assumption of random mating within a population. Even in studies of the breeding structure itself (WRIGHT 1940b, 1943, 1946), it has been assumed that, where the opportunities for mating are equal, mating is at random. The HARDY-WEINBERG formula shows that in a sexually reproducing, random breeding population where the component genotypes are equally successful in surviving, the relative frequencies of various genes in the population remain constant. Hence, the postulation of a random breeding population permits the study of the effects of different forces on a system in equilibrium.

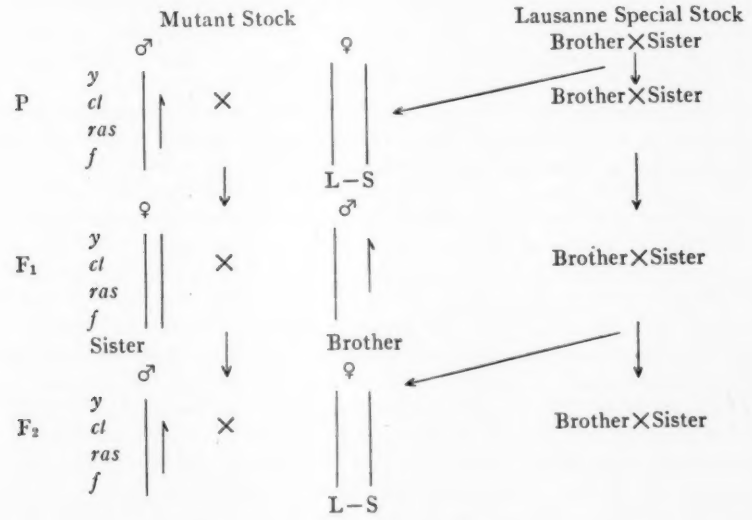
The present study was designed to ascertain whether or not mating actually was at random between the wild type and four mutant types of *Drosophila melanogaster*. If mating was found to be non-random, it was desired to determine the mating system. The results of these experiments could then serve to indicate how much of the change in gene frequency in a population is due to the effects of selective mating. Using the same mutations and their wild alleles in "population bottles" (REED and REED 1948), LUDWIN (1948) measured the variations in frequency of the genes during several generations of competition between them. A comparison of his results with the results of these experiments should indicate the relative importance of selective mating in changing the gene frequencies in populations.

[1] Adapted from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at HARVARD UNIVERSITY.

MATERIALS

The stocks used in these experiments were derived from a wild type stock of *Drosophila melanogaster* known as Lausanne Special (L-S), isolated by BRIDGES in 1938 from a strain collected at Lausanne, Wisconsin (BRIDGES and BREHME 1944). While this stock was being inbred (brother×sister) for thirty generations, a male with the sex-linked recessive genes, yellow (*y*), cut (ct^6), raspberry (ras^2), and forked (*f*) (ibid.), was crossed to an L-S female every other generation (the L-S female in each case coming from among the offspring of the single L-S pair giving rise to the generation). Thus, all the autosomes of the two stocks were soon identical, and such changes as occurred in the L-S stock during inbreeding were incorporated into the *y* ct^6 ras^2 *f* stock at the same time. The necessity for keeping the four mutants (located at 0.0, 20.0, 32.8, and 56.7) together during the crosses made it probable that sizeable portions of the X chromosome were traceable to the *y* ct^6 ras^2 *f* stock rather than the Lausanne Special.

The crosses were as follows:



and so on for thirty generations. From this stock fourteen other stocks (all the remaining possible combinations of the genes), were isolated from crossover types found in the males. In these stocks with one, two, or three genes present, there were probably portions of the L-S X chromosome incorporated. All of the flies used in the experiments came from the fifteen basic stocks thus established.

Because it was desirable to eliminate as many environmental variables as possible so that any differences in the results could be ascribed to the differing

genetic constitution of the flies, crosses were made whereby, in a particular experiment, all the flies used came from the same culture bottle, and all were offspring of the same parental types. The following cross made such a situation possible, with *ras* used as an example:

♀ gam \ ♂ gam	*ras*	Y
ras	$\frac{ras}{ras}$	*ras* Y
+	$\frac{ras}{+}$	+Y

METHODS

Studies of sexual behavior, sexual selection, and sexual isolation have, in many cases, been designed to determine whether mating is at random. Three general methods have been used. STURTEVANT (1915) used direct observation exclusively, while STALKER (1942), RENDEL (1945), and MAYR (1946b) supplemented their other data by this method. Pair matings have been used extensively by the group at the University of Texas (PATTERSON, STONE, and GRIFFEN 1940) to test sexual isolation. A comparison of the proportion of fertilized females in inter-group matings with the proportion in intra-group matings gives an indication of the degree of sexual isolation. The most common method is a multiple choice technique used widely by DOBZHANSKY et al. (DOBZHANSKY and KOLLER 1938). This method consists, essentially, of placing one kind of male with two female types. Examination of the spermathecae for the presence of spermatozoa discloses which females have been inseminated. These three techniques, observation, pair mating, and multiple choice, have been applied to test randomness of mating between species, between subspecies, races, or strains of one species, and between mutant types derived from a single strain.

Because of certain disadvantages to these techniques, others were devised which must be similar to those used to obtain the data presented in an abstract by DIEDERICH (1941). These methods permitted the study of the results of female "choice" (one female type with two kinds of males) as well as the results of male "choice" (one male type with two kinds of females). The greatest defect of the multiple choice technique described above was overcome when the study of female "preference" became possible. Henceforth, to avoid needless circumlocution, words such as "choice" and "competition" will be

used in describing the different types of matings. However, the terms are not intended to carry any of the implications ordinarily associated with them.

The flies to be used in the mating tests were removed from the stock cultures and etherized. From them were separated virgin females with the abdomen flat, the abdominal tergites not yet darkened, but with the wings fully expanded. These females were then placed at once with males from the same culture in creamers containing food. The females had, therefore, quite recently eclosed when put with the males. Although the males were generally less than twenty-four hours old, no effort was made to use only those most recently emerged. At the suggestion of DR. DOBZHANSKY, the results obtained by using these young flies were checked by aging both the males and females separately for seven days and then duplicating certain of the experiments. The unaged flies were left together in the creamers for periods ranging from 18 to 72 hours; the aged flies were separated after 2 to 7 hours, the variation in time being due to differences in the length of time needed for fertilization to occur. All experiments were carried out at room temperature.

Matings to test female "choice" were made by placing females of one type, either the mutant or the heterozygous, in a creamer containing food, with both the mutant and wild type males. The three classes were present in equal numbers, and the males, therefore, outnumbered the females two to one. The actual number of flies of each type did not exceed ten, with five of each type the number most commonly used. After the removal of the males, each female was placed in a separate creamer. The determination of the genotype of the male which had succeeded in mating with the female was made by an examination of her offspring. Since the presence or absence of an entire class depended on which male was successful, it was a relatively easy task to determine the male parent. An example may clarify this technique.

♀ $\frac{ras}{+}$ with either ♂ *ras* Y or ♂ +Y

♂ *ras* Y successful

♀ gam \ ♂ gam	*ras*	Y
ras	$\frac{ras}{ras}$	*ras* Y
+	$\frac{ras}{+}$	+Y

♂ +Y successful

♀ gam \ ♂ gam	+	Y
ras	$\frac{ras}{+}$	*ras* Y
+	$\frac{+}{+}$	+Y

The female offspring indicate the genotype of the successful male. Although females of the wild phenotype appear in both cases, the presence or absence of the recessive female class is a positive indication of the male parent. The male offspring serve as a direct check on the genotype of the mother.

A modification of this method was used in testing the effects of putting two types of mutant males in "competition" with one another. The females were all of the genotype *y ct ras f*/++++. Such a change made possible a direct comparison of the relative abilities of the mutant males to fertilize the same type of female. Only an indirect comparison was possible with the results obtained from "competition" between the wild type and the mutant males. However, since the females were heterozygous for different mutants in each set of experiments, the comparisons of the mutants thus made were not too reliable. A disadvantage to the more direct method lay in the fact that the flies came from different culture bottles.

The technique for studying male "preferences" was similar to the method just described, but two female types and only one male type were used. In no instance did the number of each type exceed ten. Any group in which more than 80 percent of the females were inseminated was not included in the data (except data with aged L-S males mating with *y*/*y* and *y*/+ females included for another purpose). The number of males was reduced in some cases because the males fertilized such a high percentage of the females. Thus, there were at least twice as many females as males in each mating creamer. After periods comparable to those cited above, the males were removed and each female was isolated in a separate creamer. The proportion of creamers in which larvae and pupae appeared was taken as the frequency of successful matings.

In case any flies died during the period in which the males and females were together, the group was discarded. If, after isolation in a creamer, a female died without leaving any eggs or larvae, the creamer was discarded (or the series if the experiment tested male "choice").

In order to eliminate the possibility that the sterility of the parents or the inviability of the zygote or early embryo gave an inaccurate picture of the number of females inseminated, the fertility of certain female types (L-S, *ct*/*ct*, and *y ct ras f*/*y ct ras f*) was tested. The *ct*/*ct* and *y ct ras f*/*y ct ras f* individuals tested were females failing to show offspring in a previous experiment. Each female was placed with five wild type males to insure fertilization. The results are shown below (Table 1). The mortality among the *y ct ras f*/*y ct ras f* females is probably due to their lower viability and their age since they were at least ten days old when these tests were begun. These experiments plus the fact that pure stocks were carried with little difficulty indicate that parental sterility and embryonic inviability did not markedly influence the results of the mating tests. In those cases in which the females produced offspring, the proof that mating had occurred was rather conclusive. It may then be assumed that the production of offspring was, in these experiments, about as reliable a criterion for the occurrence of insemination as the more direct spermathecal examination.

Flies from different species or races often require different periods of time to

TABLE 1

Tests for fertility of certain female types

GENOTYPE	NUMBER FERTILE	NUMBER STERILE	% FERTILE	NUMBER DEAD
L-S / L-S	60	0	100.0	0
ct / *ct*	22	0	100.0	0
y ct ras f / *y ct ras f*	37	3	92.5	12

reach sexual maturity. For this reason, other workers have had to separate the females and the males until all groups had reached maturity before the flies could be used in mating experiments. Such separation of the sexes is an artificial situation but is unavoidable in such cases if the results are to be significant. In these experiments, immature females were placed with the males and reached maturity in their presence. This method more nearly parallels the conditions in the population bottles and in nature.

The presence of food in the mating creamers is again a more natural situation although it might be considered a possible source of distortion in the results. Etherization of the flies may also have affected the data, but the females were so immature that, by the time mating occurred, several hours had passed since the flies had recovered from the ether.

The particular advantage in the methods employed lay in the possibility of studying matings of the female "preference" type on a basis comparable to matings of the male "preference" type. In much of the recent work (STALKER 1942; DOBZHANSKY 1944; MAYR 1946a; TAN 1946; PATTERSON et al 1947; and so forth) the latter type has been studied exclusively.

EXPERIMENTAL RESULTS

Tables 2 and 4 give the data from matings testing the "preferences" of the females. In the column headed "Successful Males," the numbers and percentages of the total number of successful males are given. In this column in table 2, the mutant males had the same mutations as the females in the same row. The column headed "Mean Hours" in all tables gives the average period that the males and females remained together.

Table 3 is a record of the data on male "preferences." The number listed under "Total Females" is the combined total of homozygous recessive and heterozygous females, which were used in equal numbers in each set of experiments. The percentages under "Fertilized Females" are the proportions of the total fertilized females.

TABLE 2

Female "Preference"

♀ GENOTYPE	♀ TOTAL	SUCCESSFUL ♂♂ MUTANT n	MUTANT %	L-S n	L-S %	χ^2	DOUBLE MATINGS	% FERTILIZED	MEAN HRS.
y/y	97	8	10.5	68	89.5	47.368	1	79.4	25.0
*y/y**	53	6	11.8	45	88.2	29.823	2	100.0	6.6
y/+	73	3	4.6	62	95.4	53.554	1	90.4	24.7
*y/+**	53	4	7.8	47	92.2	36.254	0	96.2	6.1
ct/ct	62	3	11.5	23	88.5	15.385	0	41.9	23.1
*ct/ct**	70	11	29.0	27	71.0	6.737	0	54.3	5.2
ct/+	55	6	12.8	41	87.2	26.064	1	87.3	23.0
*ct/+**	52	15	30.0	35	70.0	8.000	0	96.2	5.3
ras/ras	76	21	34.4	40	65.6	5.918	1	81.6	24.7
ras/+	68	18	31.6	39	68.4	7.737	0	83.8	24.7
f/f	87	33	54.1	28	45.9	0.410	1	71.3	22.7
f/+	75	30	55.6	24	44.4	0.667	2	78.9	24.0
y ct/y ct	41	0	0.0	16	100.0	16.000	0	39.0	37.6
y ct/++	40	0	0.0	35	100.0	35.000	0	87.5	35.8
y ras/y ras	44	3	9.4	29	90.6	21.125	2	77.3	33.2
y ras/++	33	0	0.0	31	100.0	31.000	0	93.9	34.4
y f/y f	52	9	20.0	36	80.0	16.200	6	98.1	40.9
y f/++	41	2	5.1	37	94.9	31.410	0	95.1	33.2
ct ras/ct ras	49	4	22.2	14	77.8	5.555	0	36.7	35.2
ct ras/++	49	15	38.5	24	61.5	2.077	2	83.7	36.0
ct f/ct f	47	7	30.4	16	69.6	3.522	3	55.3	35.4
ct f/++	53	9	17.0	44	83.0	23.113	0	100.0	41.9
ras f/ras f	50	8	20.5	31	79.5	13.543	6	90.0	30.1
ras f/++	50	19	45.2	23	54.8	0.381	1	86.0	30.5
y ct ras/y ct ras	48	0	0.0	30	100.0	30.000	0	62.5	45.1
y ct ras/+++	29	0	0.0	28	100.0	28.000	0	96.6	46.0
y ct f/y ct f	47	2	8.7	21	91.3	15.696	4	57.4	46.5
y ct f/+++	42	0	0.0	40	100.0	40.000	0	95.2	45.6
y ras f/y ras f	55	4	10.0	36	90.0	25.600	10	90.9	46.7
y ras f/+++	37	1	3.6	27	96.4	24.143	1	78.4	37.8
ct ras f/ct ras f	72	5	15.6	27	84.4	15.125	4	50.0	45.7
ct ras f/+++	54	12	33.3	24	66.7	3.457	2	70.4	44.7
y ct ras f/y ct ras f	68	1	11.1	8	88.9	5.444	0	13.2	29.3
y ct ras f/++++	53	0	0.0	34	100.0	34.000	0	64.2	23.8

* Males and Females aged seven days.

(a) *Single Mutants*

1. Yellow—The wild type male is much more successful than the yellow male when both are in "competition" for either the *y/y* or the *y/+* females. In table 3 it can be seen that the wild type males mated at random with either female type while the yellow males mated significantly more often with the *y/y* female than with the *y/+* female. These results agree with those of STURTEVANT (1915) and DIEDERICH (1941) in *D. melanogaster*, and RENDEL

(1945) in *D. subobscura* with the *y* gene or combinations of it. SPETT's data (1932), which indicated sexual isolation between *y* and the wild type, were not confirmed.

To eliminate the possibility that the effect of *y* was due to the visual effect of the markedly different body color, mating tests were performed in darkness. The results did not differ from those obtained in the light, and are included in

TABLE 3

Male "Preference"

♂ GENOTYPE	♀ TOTAL (50% OF EACH TYPE)	FERTILIZED ♀♀				χ^2	% FERTILIZED	MEAN HRS.
		HOMOZYGOUS RECESSIVE		HETEROZYGOUS				
		n	%	n	%			
y	104	30	69.8	13	30.2	6.721	41.3	22.6
*y**	52	18	72.0	7	28.0	4.840	48.1	5.0
L-S(*y*)	70	23	56.1	18	43.9	0.610	58.6	22.7
L-S(*y*)*	88	42	49.4	43	50.6	0.012	96.6	4.1
ct	60	15	38.5	24	61.5	2.077	65.0	23.0
*ct**	72	19	35.2	35	64.8	4.740	75.0	5.0
L-S(*ct*)	52	11	33.3	22	66.7	3.667	63.5	22.7
L-S(*ct*)*	70	14	28.6	35	71.4	9.000	70.0	4.5
ras	112	36	53.7	31	44.3	0.373	59.8	22.6
L-S(*ras*)	60	18	50.0	18	50.0	0.000	60.0	22.5
f	80	25	48.1	27	51.9	0.077	65.0	23.3
L-S(*f*)	86	25	45.5	30	54.5	0.454	63.9	23.5
y ct	144	2	16.7	10	83.3	5.333	8.3	33.8
L-S(*y ct*)	88	10	41.7	14	58.3	0.667	27.2	27.2
y ras	82	21	75.0	7	25.0	7.000	34.1	36.3
L-S(*y ras*)	78	16	43.2	21	56.8	0.676	47.4	24.1
y f	80	27	71.0	11	29.0	6.737	47.5	26.5
L-S(*y f*)	88	31	52.5	28	47.5	0.152	67.0	23.9
ct ras	98	7	18.9	30	81.1	14.297	37.8	24.6
L-S(*ct ras*)	76	9	29.0	22	71.0	5.452	40.8	30.4
ct f	84	10	34.5	19	65.5	2.793	34.5	27.5
L-S(*ct f*)	80	18	34.6	34	65.4	4.923	65.0	25.5
ras f	84	20	45.5	24	54.5	0.364	52.4	25.4
L-S(*ras f*)	74	13	36.1	23	63.9	2.778	48.6	22.2
y ct ras	96	3	25.0	9	75.0	3.000	12.5	44.2
L-S(*y ct ras*)	94	17	35.4	31	64.6	4.082	51.1	33.3
y ct f	80	7	41.2	10	58.8	0.529	21.2	33.4
L-S(*y ct f*)	82	7	24.1	22	75.9	7.759	35.4	27.9
y ras f	58	21	84.0	4	16.0	11.560	43.1	30.2
L-S(*y ras f*)	64	17	48.6	18	51.4	0.028	54.7	26.6
ct ras f	60	3	14.3	18	85.7	10.714	35.0	31.3
L-S(*ct ras f*)	68	12	30.8	27	69.2	5.769	57.4	31.4
y ct ras f	100	2	18.2	9	81.8	4.454	11.0	33.7
L-S(*y ct ras f*)	108	9	22.0	32	78.0	12.902	38.0	29.0

* Males and Females aged seven days.

the totals of table 2. Excellent agreement is shown between the results obtained with unaged and aged flies.

2. Cut—In table 2 it can be seen that the wild type males inseminated a greater proportion of the females than the cut males when the males were in "competition." Here also the low percentage of homozygous *ct* females which were fertilized should be noted. The highest percentage of fertilization for females homozygous for cut was 62.5 percent of the *y ct ras*/*y ct ras* females after an average period with the males of 45.1 hours. The lowest frequency of fertilization of any of the other female types was 64.2 percent of the *y ct ras f*/++++females after an average exposure of only 23.8 hours. This low mating frequency of the homozygous cut females appears to be responsible for many of the non-random results on male "preferences." Table 3 shows that neither the mutant nor the wild type males were as successful in mating with homozygous cut females as with the phenotypically wild type. That this low frequency was due not to sterility but to a lower frequency of mating was proved by testing the fertility of females failing to produce offspring. The results were shown previously in table 1.

Although in table 2 the results with flies aged seven days differ significantly from those with unaged flies in that cut males fertilized a higher proportion of females, mating was not random, with the wild type males still more successful than the cut males. The results in table 3 are not affected by aging.

3. Raspberry—As shown in table 2, the raspberry males were successful about half as often as the wild type males in mating with either the *ras*/*ras* or the *ras*/+ females. Table 3 shows that the male alone, either wild type or raspberry, fertilized both types of females at random.

4. Forked—The results with forked show in all cases that mating is at random. Therefore, with regard to mating the forked gene is the only one of the four that can compete with the wild type on even terms.

(b) *Combinations of Mutants*

A somewhat surprising result was the constancy of the effect of a given gene on the mating behavior in combination with other mutations. TIMOFÉEFF-RESSOVSKY (1934) found that in *D. funebris* the relative vitality of different visible mutations and their combinations can be very different. However, in the present experiments, groupings can be made where the results are similar. One such group includes *y*, *y ras*, *y f*, and *y ras f*. All are similar to *y* alone. Since neither *ras* nor *f* has a very great effect on the mating behavior, it seems probable that the similarity is due to the persistence of the effects of *y*. Another group may be made of *ct*, *ct f*, *ct ras*, and *ct ras f*, but the improved male performance in *ct ras* and *ct ras f* warrants separation into two sets. The *ras* gene seemed to improve the mating ability of *ct ras* and *ct ras f* males rather than combining with *ct* to reduce this ability. Finally, *y ct*, *y ct ras*, *y ct f*, and *y ct ras f* all show very similar patterns. The effects of *y* and *ct* on mating seem to be persistent in combination with other genes, and together their effects are combined. For example, of 215 matings in which males with both *y* and *ct* "competed" with the wild type males, they were successful only three times.

This proportion is much lower than that found with either gene alone. Therefore, with the exception of the effect of *ras* on *ct*, the genic influence seemed to be nearly additive.

(c) *"Competition" between Mutant Males*

When females heterozygous for *y ct ras f* were placed with two kinds of mutant males, the results, shown in table 4, were close to what might have been

TABLE 4

Female "Preference"

♀ GENOTYPE	♀ TOTAL	SUCCESSFUL ♂♂				χ^2	DOUBLE MATINGS	% FERTILIZED	MEAN HRS.
		n	%	n	%				
y ct ras f / + + + +	34	*y* 6	35.3	*ct* 11	64.7	1.470	0	50.0	24.8
y ct ras f / + + + +	19	*y* 0	0.0	*ras* 15	100.0	15.000	1	84.2	38.2
y ct ras f / + + + +	51	*y* 1	2.4	*f* 41	97.6	38.095	0	82.4	27.1
y ct ras f / + + + +	13	*ct* 1	8.3	*ras* 11	91.7	8.333	0	92.3	41.0
y ct ras f / + + + +	42	*ct* 8	21.0	*f* 30	79.0	12.737	1	92.8	31.7
y ct ras f / + + + +	54	*ras* 24	63.2	*f* 14	36.8	2.632	0	70.4	28.0

expected from the data on the mutants in "competition" with the wild type males. The *y* and *ct* males were unsuccessful in the presence of *ras* or *f* males. The *y* versus *ct* and *ras* versus *f* experiments showed that these types were mating at random although further data might reveal that even in these cases mating was not completely random. The success of *ras* in "competition" with *f* was unexpected because the *ras* males were not as successful in "competition" with the wild type as the *f* males.

DISCUSSION

The Mating System

The results show that the behavior of the females was primarily responsible for the occurrence of non-random mating. In the first place, there were a

greater number of significant χ^2 values in the experiments on female "choice" than in those on male "choice." Furthermore, the significant χ^2 values were much larger in the experiments on female "choice." Such results indicate that, where the male has an opportunity to mate with more than one type of female, he mates more nearly at random than is the case if the female is given the opportunity of mating with two different male types. Even the instances in which the males do not mate at random may be due to differences in the response of the females. All the significant χ^2 values in the male "choice" experiments are found in connection with the *y* and *ct* genes. The results with *ct* are due to the lower mating frequency of *ct*/*ct* females, clearly seen also in table 2. Although other explanations have been given for the results with *y* (STURTEVANT 1921), RENDEL (1945) observed that all non-yellow females refused the yellow males.

PEARSON and LEE (1903) as a definition of assortative mating stated, "While all classes of males and females find mates, certain classes of males are attracted to certain classes of females." Assortative mating may therefore be positive (homogamic—DOBZHANSKY and MAYR 1944) or negative (heterogamic). Homogamy would be characteristic of sexually isolated groups. They also defined preferential mating, "in which male and female classes with certain values of a character find it less easy to mate than other classes with different values." In these experiments, mating was not assortative but was preferential because one type of male was more successful than the other in "competition" for either type of female. It should be noted that, where mating was not random, the wild type male was always more successful than the mutant type. In those cases of male "choice" in which the wild type male mated more often with his own type females, the mutant males also were more successful with the wild type females. Under these circumstances, no sexual isolation can exist.

Theoretically, there are a great number of possible systems of breeding. The effects of these mating systems can be predicted mathematically by means of calculations of the changes in distribution and frequency of the genes in a population (JENNINGS 1916; WRIGHT 1921; HOGBEN 1946). Unfortunately, none of the formulae derived were suitable for application to the particular mating system operative in these experiments.

The nature of this mating system can be understood from a consideration of the data. It may be concluded that practically all of the females in a population will, in time, be fertilized. This conclusion is reached because of numbers of experiments on male "choice" in which practically all females were inseminated by a smaller number of males, necessitating discard of the series, and the high percentage of fertilization in the experiments on female "choice." Even in the case of females homozygous for cut, it is evident from the data in table 1 that all would ultimately be fertilized although their reproductive rate would be lower than normal because of the time lapse prior to fertilization. Under natural conditions, the insemination of the females is also close to 100 percent (PATTERSON, MCDANALD, and STONE 1947). In their table 1, of 547 females of *D. melanogaster* collected in the wild, 95.6 per cent had been inseminated. Of twelve other species listed, at least two-thirds of the females con-

tained sperm, and ten of the twelve species had 84 percent or more of the females inseminated.

Furthermore, it must be concluded that, given the opportunity, the males will mate more often than the females. The results indicate that the males generally mated more than once during the period of exposure. The data with seven-day-old L-S males and $y/+$ and y/y females show that in an average period of just 4.1 hours, the males inseminated 96.6 percent of twice their number of females, an average of 1.93 matings per male in 4.1 hours. Not shown are the 79 series where the fertilization of more than 80 percent of the females made the results valueless in determining the nature of the male "preferences."

On the other hand, if it is assumed that the appearance of two classes of offspring from a female gives a rough indication of the frequency of double mating, the females seldom mated more than once. Because the detection of double mating by the heterozygous females is more difficult, a better estimate is gained from the recessive females. From table 2 it can be calculated that only 5.9 percent of the homozygous recessive females mated with both male types. Although double mating with the same male type can not be detected, nevertheless it is doubtful that the incidence of double matings including this type would rise above 15 or 20 percent. The frequency of male mating contrasted to the fewer matings by the females would lead to the fertilization of several females by one male while another less successful male might not mate at all.

From these conclusions, the mating system can be deduced. As each female becomes sexually mature or sexually receptive in the population bottle, a number of males will be ready to court and copulate with her. This surplus of males then becomes, in a broad sense, a competing group, the female mating with only one of several available males. Therefore, one male may mate with several females while another male fails to inseminate any. The males, furthermore, seem to mate at random. Random mating in the male "choice" experiments is prevented only by two factors: lack of mating success by y males with females other than y/y, and the low mating frequency of the homozygous cut females.

The mating system may therefore be considered to be the partial sex-limited selection of a sex-linked gene. The fertilization of all females means that, in their case, there is no differential elimination of the gene because of selective mating. However, because of the differential mating success of the males and the fact that the males are essentially polygynous so that one male fertilizes as many females as possible, there is a selective elimination of one genotype among the males. Hence, these experiments indicate that the elimination of the recessive sex-linked gene occurs in the males. There should also be a higher proportion of the presumably better adapted wild phenotype than is possible with random mating. The results obtained by LUDWIN (1948) in the population bottles confirm this conception of the mating pattern.

Even though the causes are obscure, the results with many different species have shown that mating is seldom random. Extensive experiments with Drosophila demonstrate that selective mating will not only maintain species

differences but will also affect the genetic structure of populations (STURTEVANT 1915; LANCEFIELD 1929; DIEDERICH 1941; DOBZHANSKY and MAYR 1944; TAN 1946; etc.). Findings such as these are more basic in their implications than any dispute as to the causes of this non-random mating, for they form the premise on which DARWIN originally constructed his theory of sexual selection. If mating within a species is not at random, it must follow that the mating process is a very definite and important part of the selective forces acting on each generation. Furthermore, non-random mating is not restricted to Drosophila, but occurs in a variety of species, from the Protozoa (JENNINGS 1911) to man (ROBINSON 1934). Its causes are varied, and its effects range from negligible to extremely large. In any study of the selective forces acting on a sexually reproducing population, the assumption of random mating without supporting evidence is unreliable.

Courtship Behavior in Drosophila

A number of authors have published descriptions of the mating behavior of different species of Drosophila (STURTEVANT 1915, 1921, 1942—*D. melanogaster* and numerous others; STALKER 1942—*D. virilis;* RENDEL 1945– *D. subobscura;* MAYR and DOBZHANSKY 1945, MAYR 1946b—*D. pseudoobscura, D. persimilis;* SPIETH 1947—*D. willistoni* species group; SEARS 1947—*D. quinaria* species group). All these observations showed that, on the whole, the mating behavior within the genus was rather similar, with minor variations occurring among the different species. The descriptions may briefly be summarized from STURTEVANT (1915), MAYR (1946b), and SPIETH (1947) by a consideration, first, of the different elements in the male behavior. The courting activities of the males are well defined so that it is easy to identify a courting male. Most of the following actions are common to most males of the genus Drosophila: vibrating, waving, scissors actions (characteristic wing movements); circling of the female by the male; licking; tapping, uppercutting, and stamping (leg movements).

The female behavior is much simpler. If unreceptive, the female will refuse the male by "decamping" (SPIETH 1947), by flicking the wings, by depressing or raising the abdomen, by extruding the genitalia so that the male can not mate, or simply by ignoring the male. If receptive, the female stands still, turns the tip of the abdomen toward the male, and partly spreads the genitalia in the "invitation display." A ready male will copulate at once with the female, but the male can not mate if the female is unreceptive.

Animal psychologists (for instance LORENZ 1935; TINBERGEN 1939, 1942) who have studied mating behavior have established that most courtship patterns, especially in vertebrates such as birds, fish, and so on, are composed of a series of actions and reactions between the male and the female. A stimulus, whatever its nature, from one individual, elicits a response from the other. The response, in turn, if it is positive, acts as a new stimulus to the first individual, evoking from it still another response. In this light, courtship may be regarded as a chain of reactions, or even a chain reaction, leading to progressively higher states of nervous excitation which ultimately culminate in copula-

tion. A failure by either individual to respond positively at any point in the chain would delay the progress of the courtship or cause it to be broken off. Such a concept is of great value since complex behavior patterns can be analyzed in terms of stimuli and responses without resorting to more complex terms such as "recognition" or "preference."

Such a pattern of behavior undoubtedly exists among many forms, and particularly among birds where it has been most closely studied. However, even though the more recent authors (MAYR 1946b; SPIETH 1947; KING 1947; STREISINGER 1948) have made their observations with this theory as a basis, the evidence for its existence in Drosophila is not clear.

In an extensive review on sexual selection among the insects, RICHARDS (1927) described and discussed what he termed "female coyness" in insects, the reluctance of the females to mate at once. The "coyness" of the female, common to many species, was considered to be the cause for the elaborate courtship. According to HUXLEY (1938) the display in many of the lower forms, including Drosophila and other insects, is unilateral, the courtship by the male engendering a readiness to mate in the female. A recollection of the description of courtship above will call to mind that all of the positive actions were made by the male up to the point at which the receptive female, by spreading the wings and genitalia, permits copulation. Prior to this final acceptance, the female shows no activities which might serve as stimuli to the male. If a succession of actions and reactions between male and female were actually the pattern necessary, it is strange that the male courting activities ("circling," "vibrating," etc.) are so readily recognized by the observer, but no comparable behavior is seen in the female. If the female is unreceptive, her actions in refusal are more positive than if she is receptive.

A succession of progressive stimuli from the female does not seem to be necessary to produce the copulatory state in the male. Males, apparently driven by an innate sexual urge, will initiate courtship with any individual, male or female, of the same or closely related species. Several authors (as for example RENDEL 1945, WALLACE and DOBZHANSKY 1946) have observed that males will start to court other males. In the *quinaria* species group, SEARS (1947) observed that in interspecific crosses the females showed little or no interest in the males or were even antagonistic while the males courted the females actively and even attempted to copulate despite the hostility of the females. MAYR (1946b) noted an absence of species discrimination in courting males of *D. persimilis* and *D. pseudoobscura*. Furthermore, he stated that males will mate without the "invitation display" from the female. Further evidence has been presented by STREISINGER (1948). He found that males of *D. melanogaster* inseminated etherized "unconscious" females of *melanogaster* and *persimilis* at random, but that, of the unetherized females, only *melanogaster* females were inseminated. Similarly, males of *D. pseudoobscura* inseminated etherized *pseudoobscura* and *persimilis* females at random, but, of the unetherized females, 90 percent of those inseminated were *pseudoobscura*. On this evidence, it is difficult to justify his statement that "Copulation is the result of a chain of interdependent stimuli and reactions between the male and the

female." There must be some constant non-specific stimulus from the females which serves to attract the males. This stimulus alone is enough for the males to complete copulation with the etherized females. The male seems to require no other stimulus or response on the part of the female. However, the unetherized females must require a series of stimuli from the males before they permit copulation. Furthermore, these stimuli from the males seem to be a characteristic of the species, in contrast to the apparent non-specific nature of the stimulus from the females. The evidence is strong that the males actually have no preferences in mating with the females, and that deviations from random mating in experiments on male "choice" are due to differing responses by the females.

All these results do not seem to fit the idea of a succession of stimuli and responses causing mutual stimulation. Rather it seems that the male attempts to stimulate the female to the point where she allows coition. Hence, in the case of Drosophila, HUXLEY's idea of unilateral stimulation appears to be more nearly accurate.

This conclusion takes on particular importance because of the recent work on sexual isolation to be discussed below. If it is assumed that mating results from "a chain of interdependent stimuli and reactions," significant results are obtainable from experiments testing either male or female "choice." If, however, the role of the male is secondary to that of the female in the occurrence of mating, experiments on female "choice" should be far more revealing than those on male "choice." Since it is becoming increasingly clear that the males court and mate at random unless the female responses differ, the continued use of male "choice" techniques in the study of sexual isolation holds less promise of a satisfactory solution to the problem than the use of techniques measuring female "preferences."

Sexual Isolation

The recent interest in sexual isolation has resulted in considerable experimental data. Practically all of these data have been obtained by the multiple choice technique previously described, in which a male type is placed with two female types and a spermathecal examination reveals which females have been inseminated. This method makes possible the study of the "preferences" of the males. It is unfortunate that this particular method has been so extensively used because, from the data and the foregoing discussion, it is clear that the males are much more apt to mate at random than the females.

The conclusions from the present study make it worthwhile to attempt to interpret some of the previous data on sexual isolation. STALKER (1942) formulated an isolation index as a measure of the sexual isolation between different groups when the multiple choice technique is used.

$$\text{Isolation Index} = \frac{\%\ \text{Homogamic} - \%\ \text{Heterogamic matings}}{\%\ \text{Homogamic} + \%\ \text{Heterogamic matings}}.$$

A positive value indicates isolation, values near zero no isolation, and a nega-

tive value a preponderance of heterogamic mating, with the limits +1 and −1.

In Drosophila, two kinds of selective mating have been observed. In interspecific crosses, (such as LANCEFIELD 1929, DOBZHANSKY and KOLLER 1938), true sexual isolation or a preference for homogamic mating has been found to be the rule. For example, groups A and B are sexually isolated if males of A mate chiefly with A females, and B males with B females. However, the efforts to study the origin of this isolation by working with intraspecific crosses of strains, races, or subspecies (DOBZHANSKY and MAYR 1944; DOBZHANSKY and STREISINGER 1944; PATTERSON, MCDANALD, and STONE 1947) have produced some "unexpected" results due to "one-sided mating preferences" (DOBZHANSKY 1944). In this situation, males of A mate more often with A than with B females (positive isolation index), but B males either mate at random (isolation index close to zero) or mate more often with the A females (negative isolation index). In some cases, these negative isolation indices, indicative of a "preference" for heterogamic mating, have been as large as −0.5. (Theoretically, males of both types could "prefer" heterogamic mating, but no case of this type is known.)

A most interesting fact is that the results showing "one-sided mating preferences" in different strains correspond exactly with those obtained with mutant types. Here, too, in many instances, males of mutant type A mate more often with A than B females, but males of mutant type B either mate at random, or mate more often with the A females (e.g. the data with yellow and cut). In these experiments, negative isolation indices are also common. The data on sexual isolation give no idea of the "preferences" of the females, but rather of the relative ease with which they mate, since the females were not offered a "choice" between two males. The males, showing little or no discrimination, mate with the females most responsive or least resistant to their courtship

Viewed in this light, the data of DOBZHANSKY and STREISINGER (1944) with geographic strains of *D. prosaltans* become more understandable. Among seven strains of *D. prosaltans*, they discovered true sexual isolation in a few crosses and "one-sided mating preferences" in many others. A hierarchic series was made up, by which it became generally possible to predict which of any two female types would be inseminated more often. It seems more reasonable to assume that there are constant differences among the females rather than a hierarchy of sexual "preferences" by the males. The latter assumption demands that the males of many different strains exhibit identical "preferences," which seems less likely than that the results are due to invariable differences in the responses of the females.

In all intraspecific matings between different groups, two quite different mating patterns may occur, true sexual isolation or a "one-sided mating preference." If the factors causing both these patterns acted in the same cross, the data would be very difficult to interpret. However, it seems unlikely that the "one-sided mating preferences" represent the first step along the road to true sexual isolation (DOBZHANSKY 1944). The similarity of the results with mutants and with strains suggests physiological differences not directly related to mat-

ing behavior (STURTEVANT 1915; DOBZHANSKY 1944). This hypothesis makes it possible to interpret much of the information which has been accumulated on sexual isolation. Until more is learned from experiments on female "choice," there seems to be no reason to interpret the data otherwise.

Experiments on temperature effects by MAYR and DOBZHANSKY (1945) furnish data of interest in this connection. Ordinarily, true sexual isolation exists between *D. pseudoobscura* and *D. persimilis* (MAYR 1946a). Thus, the seeming discrimination of the *persimilis* males against their own females which appears at low temperatures is surprising but perhaps can be explained. *D. persimilis* lives in cooler habitats and remains, therefore, more active at 18° and 16½°C than does *D. pseudoobscura*. The *persimilis* males, which MAYR (1946b) observed to court either female indiscriminately, must be able to copulate with the *pseudoobscura* females more easily than their own at low temperatures since the *pseudoobscura* females are no longer active enough to repulse them. The *persimilis* females, however, remain active at the lower temperatures and are still able to repulse both *persimilis* and *pseudoobscura* males. In this case, the reciprocal aversion of true sexual isolation has been changed into a "one-sided mating preference" by a change in the laboratory conditions.

These two mating systems complicate any experiments with hybrids, but such tests may furnish certain information. MAYR (1946a) believes that the F_1 hybrid females from *D. pseudoobscura* and *D. persimilis* crosses mate very readily because they are more vigorous than the parental types. TAN's similar results (1946) with weak back-cross hybrid females from the same two species make this interpretation questionable. MAYR's data show that the *persimilis* males inseminated a higher percentage of hybrid females than of *persimilis* females. The *pseudoobscura* males, however, inseminated a lower percentage of hybrid females than of *pseudoobscura* females. These results might be expected since the *persimilis* females do not mate as readily as the *pseudoobscura* females. Thus it seems more likely that the F_1 hybrid females are intermediate to the parental females in receptivity.

In this connection, it is rewarding to examine further TAN's data (1946). Although most of the individual χ^2 values in tables 3, 4, 5, 6, and 7 are not significant, the χ^2 for the total in each table is significant. In every case, the hybrid females are mated with more often than the pure type. The most probable underlying cause for this result is the reduction in vigor of these back-cross hybrid females. TAN suggests that the combination of two non-allelic dominant gene complexes, one from each species, is responsible. This explanation is open to question since, if such complexes were present, the hybrid females should be equally acceptable to a male of either type, but not more so than the females of his own type, and mating would be at random. Furthermore, since it is possible to cross the two species, it is unlikely that they differ greatly in their sexually attractive features. These objections argue against the genetic explanation offered by TAN.

In TAN's table 5, *persimilis* males mate much more often with hybrid females than with *pseudoobscura* females. It is a question whether this mating

occurs because of genetic factors causing the hybrids to be more desirable, or whether it is due to the greater weakness of the hybrids with an unbalanced genetic system. The latter seems more probable since *persimilis* males in table 6 are shown to mate more often with hybrid females than their own females. Furthermore, the greater the number of *pseudoobscura* chromosomes present in the hybrid, the more pronounced is this tendency.

All this evidence suggests that the "one-sided mating preferences" of mutants and of different strains are due to the same underlying cause. The probability is that physiological differences not directly associated with the mating process are responsible for the results. This hypothesis furnishes an adequate explanation for most of the data although further knowledge may cause it to be modified. Undoubtedly, the most likely source of knowledge concerning the origin of sexual isolation lies in experimentation on female "choice."

SUMMARY

1. Matings between the wild phenotype and all of the combinations of four mutants (*y*, *ct*^{6}, *ras*^{2}, *f*) of *Drosophila melanogaster* have been studied. The "preferences" of both the males and the females were tested.

2. Deviations from random mating occurred more often and were greater in the experiments on female "preference" than in those on male "preference."

3. With a "choice" of two female types, the males mated at random except when homozygous *ct* or *y* females were involved.

4. The *y*, *ct*, and *ras* males in "competition" with the wild type males inseminated significantly fewer females than the wild type males. Mating between *f* and wild type flies was at random.

5. In combination, the effects of the mutations persisted, except in some cases involving *ras*.

6. The mating system in populations with these genes and the wild type alleles would be the partial sex-limited selection of a sex-linked gene. Since practically all of the females would be fertilized, a selective elimination of the mutant genotype would occur only among the males.

7. The assumption of random mating can be justified only by supporting evidence. Even in these experiments, the effects of selective mating ranged from negligible to very large.

8. The similarity between the "one-sided mating preferences" found in mating experiments with mutants and in those on sexual isolation suggests that the same cause underlies both. These "one-sided mating preferences" do not seem indicative of incipient sexual isolation, but are probably due to causes not directly related to the mating process.

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INBREEDING IN A CLOSED FLOCK[1]

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INTRODUCTION

CONSIDERABLE interest is now centered on the question of inbreeding as applied to poultry flocks bred for high fecundity. Very recently SHOFFNER (1948) has presented data on several lines of birds some inbred as much as 60 percent and others approaching zero. SHOFFNER's data support the general thesis that close inbreeding tends to reduce hatchability, decrease egg production and to retard sexual maturity. He found no effect on body weight or egg size.

Among other things, LERNER and HAZEL (1947) made a study of the amount of inbreeding that occurred in a flock of White Leghorns over a 12-year period. Matings had been planned to avoid inbreeding and some outside stock was brought in during the first nine years of the experiment. The flock of 1935 had an average coefficient of inbreeding of 1.5 percent. There was an increase in the average coefficient on successive years up to a mean of 8.1 percent in 1941. The actual increase in the inbreeding coefficient amounted to about 2.1 percent for each generation. In this flock inbreeding was somewhat higher in the lower producing birds.

Information is desirable concerning the amount of inbreeding that actually occurs in a closed flock where selection is based on fecundity characters and where matings of closely related birds are constantly avoided.

MATERIALS AND METHODS

Rhode Island Reds bred for high fecundity since 1916 were used for study. The last five generations have been considered and pullet year performance records were used exclusively. The character and extent of the data are recorded in table 1. All stock was pedigreed.

Table 1 shows that 57 sires mated to 200 dams gave 1874 daughters for the laying houses. From the total of 1874 daughters only 1293 gave normal 365-day egg records. The rest either died or had egg records that were considered to be abnormal. The table also shows that the mean egg production of survivors did not change in five generations and that a modified type of selection of breeders may be necessary to raise the production level.

The mean degree of inbreeding in each generation was calculated by multiplying WRIGHT's inbreeding coefficient for each family through five ancestral generations by the number of daughters housed, adding these together and dividing by the total number of daughters concerned. Tracing the pedigrees back further would have been wasted time because the inbreeding coefficient

[1] Contribution No. 686 from the MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION.

TABLE 1

Data on inbreeding

GENERATION	NO. OF SIRES	NO. OF DAMS	DAUGHTERS HOUSED	DAUGHTERS COMPLETING	DEAD OR ABNORMAL PERCENT	INBREEDING COEFFICIENT	EXPECTED COEFFICIENT RANDOM MATING	MEAN PRODUCTION
1942	6	24	201	146	27	5.33	2.60	233
1943	10	27	265	162	39	2.85	4.31	232
1944	12	39	386	286	26	3.73	5.67	230
1945	14	56	515	365	29	4.37	6.78	224
1946	15	54	507	334	34	4.07	7.84	232
Totals	57	200	1874	1293				

would have increased insignificantly. Table 1 records the inbreeding coefficients of each generation, the number of daughters housed, number completing the year, percent dying or giving abnormal records and mean production.

Table 1 indicates that there was a low degree of inbreeding in each generation and that there was no tendency for the amount of inbreeding to increase in successive generations. The percentage of pullets dying or showing abnormal egg records was not consistent with the degree of inbreeding in the flock as a whole.

The calculated coefficients of inbreeding by WRIGHT's (1931) formula for a reasonably large population mated at random were higher than the actual

TABLE 2

Degree of inbreeding and egg production

INBREEDING COEFFICIENT	NO. OF FAMILIES	TOTAL NUMBER COMPLETING	AVG. FAMILY SIZE BIRDS COMPLETING	MEAN WEIGHTED PRODUCTION
0	10	64	6.4	239
.01– 3	101	701	6.9	232
3– 5	42	255	6.1	235
5– 7	15	93	6.2	222
7– 9	13	85	7.1	217
9–11	3	17	5.7	212
11–13	1	4	4.0	195
13–15	5	30	6.0	219
15–17	2	10	5.0	223
17–19	1	1	1.0	132
19–21	1	2	2.0	190
21–23	1	5	5.0	228
23–25	0	—	—	—
25–27	5	26	5.2	209
Totals	200	1293		

coefficients in the last two generations. This fact indicates that by careful selection of breeders on the pedigree basis, inbreeding may be held at a low level without introducing new stock.

INBREEDING AND EGG PRODUCTION

Inbreeding ranged from zero to slightly over 25 percent in the different families. In table 2 the total population in five generations is classified with respect to degree of inbreeding.

Table 2 indicates that mean annual egg production was highest in the families with no inbreeding. When the inbreeding coefficient was below five percent, the decline in egg production was not very great. When the inbreeding coefficient was above five percent there was a significant decline in mean egg production. In general, there is some evidence of declining production with higher degrees of inbreeding, but there are exceptions.

EFFECTS OF INBREEDING ON FECUNDITY CHARACTERS

Such fecundity traits as age at sexual maturity, incidence of winter pause, winter intensity, egg weight to January 1 and persistency have an important relation to the number of eggs laid. In table 3 the possible effect of degree for inbreeding on these traits is considered.

TABLE 3

Effects of inbreeding on fecundity characters in daughters

PERCENT INBRED	MEAN AGE AT FIRST EGG		PERCENT WITH WINTER PAUSE		MEAN WINTER CLUTCH		MEAN EGG WT. TO JAN. 1		MEAN ANNUAL PERSISTENCY	
	No.		*No.*		*No.*		*No.*		*No.*	
0	86	181	81	38.3	81	3.5	82	54.2	64	353
.01– 3	923	196	857	40.3	861	3.3	860	55.4	701	352
3– 5	357	200	322	45.3	324	3.3	328	55.3	244	353
5– 7	135	198	126	52.4	126	2.9	128	55.8	104	347
7– 9	123	199	113	42.5	114	2.9	116	56.0	85	349
9–11	24	194	21	57.1	21	2.7	21	55.3	17	344
11–13	6	187	4	75.0	4	2.9	3	56.3	4	349
13–15	47	196	44	36.4	44	3.2	44	53.4	30	353
15–17	11	190	10	60.0	10	3.0	10	53.2	10	337
17–19	1	219	1	100.0	1	1.5	1	54.8	1	286
19–21	5	181	4	100.0	4	2.8	4	49.6	2	321
21–23	9	235	9	77.8	9	2.3	8	56.0	5	348
23–25	—	—	—	—	—	—	—	—	—	—
25–27	36	215	35	62.9	35	2.4	33	53.88	26	339

The data in table 3 suggest that degree of inbreeding does not greatly affect the age at sexual maturity. There was a slight tendency for maturity to be slowed up by a more intimate degree of inbreeding but the inbred birds could not be considered as late in sexual maturity. Certainly the effects of the degree

of inbreeding concerned in this flock were not as great as the effects of recessive genes *e* and *e'* (HAYS 1945).

The incidence of winter pause was lowest in the non-inbred pullets and there was a significant increase in pause incidence as inbreeding became more intense.

Winter intensity measured by the mean winter clutch size was definitely affected by inbreeding. Here the regression approached linearity. From the data available, there appears to be a consistent decline in clutch size as the inbreeding coefficient increased. In view of this fact, it would appear that inbred birds are likely to lay fewer eggs because of lower intensity.

TABLE 4

Degree of inbreeding and laying house mortality

DEGREE OF INBREEDING	DAUGHTERS HOUSED	DAUGHTERS COMPLETING	% MORTALITY
0	88	64	27.2
.01– 3	978	701	28.3
3– 5	382	255	33.2
5– 7	134	93	30.6
7– 9	132	85	35.6
9–11	24	17	29.2
11–13	6	4	33.3
13–15	47	30	36.2
15–17	12	10	16.7
17–19	1	1	0
19–21	5	2	60.0
21–23	10	5	50.0
23–25	—	—	—
25–27	40	26	35.0
	1859*	1293	

* This total includes only families in which some daughters completed normal egg records.

Our data furnish no evidence that inbreeding affects egg size adversely. This is in agreement with the observations of SHOFFNER (1948) and many other investigators.

Degree of inbreeding appeared to have no effect on persistency of laying. Since persistency or length of the laying year has been shown to be the most important single character affecting annual egg production, it is evident that inbreeding does not reduce egg production by shortening the laying year.

In general, the data in table 3 appear to indicate that the decline in egg production associated with inbreeding is attributable largely to lowered intensity of laying and to a lesser extent to a higher incidence of winter pause.

INBREEDING AND MORTALITY

If the degree of inbreeding affects the laying house mortality, this evidence should appear in table 4 which includes the total population.

With few exceptions mortality tends to increase as inbreeding increases. Unfortunately the number of daughters in the higher inbred groups from 10 percent upward is too small for statistical reliability. With adequate numbers of daughters ranging from 0 to 10 percent inbred, there appears to be a slow but consistent increase in mortality as the degree of inbreeding increased.

DISCUSSION AND SUMMARY

In a completely closed flock of Rhode Island Reds a very low degree of inbreeding appeared in five generations. The maximum occurred in the first generation at 5.33 percent inbreeding and there was something of a decrease in degree of inbreeding through the period. These data do furnish evidence that a flock of from 200 to 500 pullets may be produced in a closed flock over a period of time without close inbreeding provided the stock is pedigreed so that close matings may be avoided. In fact the maximum degree of inbreeding was 5.33 percent while 6.1 percent inbreeding results from single first cousin matings.

Our data suggest that there is likely to be a decline in egg production when matings equivalent to single first cousin matings are made. Matings as close as a single generation of half-brother sister matings (12.5 percent inbreeding) may affect egg production adversely. Only five families showed a degree of inbreeding comparable with a single generation of full brother sister matings (25 percent inbreeding). The mean egg production of these daughters was 209.

As far as fecundity traits are concerned, the degree of inbreeding has some effects. Age at sexual maturity is slightly increased by closer inbreeding. The incidence of winter pause rises with increased inbreeding. Winter intensity appears to be very significantly reduced by inbreeding. Egg size is not affected but persistency may be very slightly reduced by inbreeding.

A gradual decline in viability accompanies an increased degree of inbreeding. Mean mortality was about 27 percent in the non-inbred group compared with a value of 35 percent when the inbreeding coefficient rose to 12 percent or higher.

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THE HERITABILITY OF ALL-OR-NONE TRAITS: VIABILITY OF POULTRY

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ONE of the fundamental steps in any analysis of the inheritance of quantitative characters is the partitioning of the total phenotypic variance of the trait under study into its genetic and environmental portions.

The various techniques for this purpose for characters showing continuous or graded types of variation are well known (see WHATLEY 1942). They are based on methods of variance and correlation analysis derived in the main from the studies of WRIGHT (1921), although many phases of the general problem were attacked earlier by WRIGHT (1917) and several other investigators (among them WEINBERG 1909; FISHER 1918 *et al.*). In the case of characters whose phenotype is expressed in an all-or-none manner these methods do not directly apply. However, special techniques based on the inverse probability transformation have been used in this connection by WRIGHT (1934a, 1934b, 1943), while more recently LUSH, LAMOREUX and HAZEL (1948) considered the problem in a more direct relationship to applied animal breeding practice. Reference to the methodology and results of this investigation will be made in the course of discussion. It may also be noted that at least one other attempt at what may be interpreted as the estimation of heritability of an all-or-none trait (multiple births in cattle) is on record, an attempt which, however, did not consider fully the difficulties of application of the analysis of variance to binomial data (KORKMAN 1948).

It is actually possible to derive a simple technique for dealing with all-or-none data by considering certain properties of the "degree of heritability," which for quantitative characters may be defined as the proportion of the phenotypic variance due to additively genetic differences between individuals. The purpose of this communication is to describe a method of approximate determination of the degree of heritability of traits expressed in an all-or-none manner, and to discuss briefly the significance of the results obtained. The derivation presented is applied to raw percentage data as contrasted with similar formulas developed by WRIGHT (*loc. cit.*) and applied to transformed data.

The problem was suggested by the junior author who also supplied the material, the general solution and actual analysis having been carried out by the senior author, while the responsibility for the presentation and discussion is shared by both.

MATERIAL

The data used in the present study consist of the 18-months mortality records for 12 years of a flock of Single Comb White Leghorns, maintained at the UNIVERSITY OF CALIFORNIA in Berkeley. Detailed descriptions of the selection procedures used in breeding for egg production and other pertinent information regarding the flock have been presented elsewhere (TAYLOR and LERNER 1938; LERNER and HAZEL 1947; DEMPSTER and LERNER 1947). It may be sufficient to say here that the main efforts in the breeding program were directed towards increasing the first-year egg *production index* (average hen-housed egg production), which represents a combination of egg production, viability, and the interaction between them.

The only other point in connection with the data which requires comment is the fact that all of the birds which died in the first laying year were subjected to post-mortem examination. The birds which were not kept after the end of the first laying year (approximately 18 months of age) were killed and autopsied. Any animals in this category which exhibited significant pathological lesions were included in the group of dead rather than surviving birds. The autopsies were performed by the Department of Veterinary Science of the UNIVERSITY OF CALIFORNIA in all of the years, except for a period during the war, when some were carried out by members of the Division of Poultry Husbandry. In addition to the study of the heritability of total first year mortality, two specific types of pathological disturbance are also discussed. The first is lymphomatosis, a complex of neoplastic diseases of particular importance in the commercial field (see TAYLOR *et al.* 1943, for a report on breeding investigations on resistance to lymphomatosis in this flock), while the second may be termed *reproductive* disorders, consisting of various types of breakdown of the genital system. This type of pathological involvement represented the major single cause of loss in the flock under study (1083 from a total of 5064 birds involved) while lymphomatosis accounted for 411 deaths from a total of 2107 deaths recorded.

THE DETERMINATION OF HERITABILITY

The change in the average genotype for a given character in successive generations under selection is a function of the heritability and the selection differential. Hence, if this change in successive generations and the selection differential are known, it is possible to estimate the heritability. In other terms the heritability is the regression of genotype on phenotype and can be determined with equal accuracy, whether or not the distribution of the independent variable (in this case, the phenotype) is normal.

With respect to mortality in a population in which no artificial selection is applied, there exists a natural selection differential for viability since birds with a genotype for low viability will have a lower chance of surviving to become parents of the next generation than the birds with a genotype for high viability. The improvement in the average genotype by natural selection may easily be calculated and allows a ready determination of the degree of heritability of mortality.

Let the genotypic values for viability of members of one generation be $p_1, p_2, \cdots, p_n$, with the mean $\bar{p}$ and the variance σ_p^2. That is to say that p_1, p_2, etc. are the probabilities of survival of the corresponding genotype under the postulated given array of environmental conditions to which the birds are exposed. The phenotype for survival of the m-th bird will then be p_m+e_m where e_m is the environmental component, and includes all factors determining the phenotypic value with the exception of the genetic one. This phenotype will necessarily take the value of either 0 or 1. The mean genotype of survivors, which may be designated as ${}_1\bar{p}$ to distinguish it from the mean genotype of all birds ($\bar{p}$), is then

$$ {}_1\bar{p} = \frac{\sum_{m=1}^{n} p_m(p_m + e_m)}{\sum_{m=1}^{n} (p_m + e_m)}. $$

Since the expected values of

$$ \sum_{m=1}^{n} e_m \quad \text{and} \quad \sum_{m=1}^{n} p_m e_m $$

are equal to zero, if birds are kept under conditions in which there is no correlation between genotype and environment, we have

$$ E({}_1\bar{p}) = \frac{E\left(\sum_{m=1}^{n} p_m^2\right)}{E\left(\sum_{m=1}^{n} p_m\right)} $$

where E denotes the "expected value" for the expression in parentheses.

The expected gain over the previous generation is[1]

[1] It may be helpful to give a broader form of derivation in addition to the above. If g_m is taken as the total genotypic value of the m-th bird, p_m as its additively genotypic value, and e_m as the environmental contribution, the additive genotype of the survivors is $\frac{\sum p_m(g_m+e_m)}{\sum(g_m+e_m)}$. The expected values of $\sum p_m e_m$ and of $\sum e_m$ will by definition be zero. Hence, the additive genotype of the survivors and the mean additive genotype of the next generation is $\frac{\sum p_m g_m}{\sum g_m}$. The improvement in the mean genotype then is $\frac{\sum p_m g_m}{\sum g_m} - \bar{p}$. Since $\bar{g}$ here equals $\bar{p}$, the expectation for the gain is $\frac{E[g_m(p_m-\bar{p})]}{\bar{p}} = \frac{\sigma_p^2}{\bar{p}}$, as given in the text.

$$E({}_1\bar{p} - \bar{p}) = \frac{E\left(\sum_{m=1}^{n} p_m^2\right)}{E\left(\sum_{m=1}^{n} p_m\right)} - \bar{p}$$

$$= \frac{n(\bar{p}^2 + \sigma_p^2)}{n\bar{p}} - \bar{p}$$

$$= \frac{\sigma_p^2}{\bar{p}} .$$

The phenotypic selection differential applied in this case is then $1 - \bar{p}$, being the difference between the phenotype of the survivors (taken as unity) and the mean phenotype of the population. Therefore,

$$\text{heritability} = \frac{\text{genetic improvement}}{\text{phenotypic selection differential}}$$

$$= \frac{\sigma_p^2}{\bar{p}(1 - \bar{p})} .$$

This gives an expression for the heritability in terms of the genotypic variance for viability and the mean viability, an expression which also can be derived more directly (compare WRIGHT 1943).

The determination of the genotypic variance is possible by a consideration of the application of the analysis of variance to binomial data. The direct analysis of the data, in which a death or a survival is considered as a separate observation with possible values of zero or of unity, has theoretical objections, since the variance is not independent of the mean, thus violating one of the fundamental requirements of variance analysis. This difficulty can be overcome by the arc-sine transformation. However, the genotypic variance in our data proved to be so small that the direct method could be used on the raw data.

There is, of course, the general question as to the significance of the degree of heritability derived from the raw data as compared with that obtained by using one or another type of transformation. We shall not enter the various aspects of this problem here except for the discussion of the probit transformation in connection with the variation in heritability related to mortality level (see below). The reason why this discussion is made in specific reference to the "probit" rather than to the "inverse probability" transformation of WRIGHT (1926), which is essentially the same, lies in the fact that LUSH, LAMOREUX and HAZEL (1948), whose results are being referred to, have couched their analysis in probit terms.

In data on poultry, the estimation of genotypic variance is greatly simplified as compared to the case of mammals by the relatively high numbers of offspring each bird may leave, especially in the case of sires. In the data here considered, each dam had an average of about eight offspring and each male an average of

about 40 (after elimination of smaller classes as noted below). It then becomes possible to carry out an analysis of variance between the offspring of males within years, and between the offspring of females within males within years, and thus obtain an estimate of the component of variance due to the differences between classes, much as has been done previously in the case of continuously distributed characters (for instance by WHATLEY 1942).

Consider, for instance, the analysis between the offspring of males in a given year. The table of data will then read as follows:

Offspring of male	Total	Surviving
I	n_1	a_1
II	n_2	a_2

etc. with a total of N males.

The total of survivors in the offspring of male I can then be considered as the total sum of n_1 observations (that is, a_1 taken as unity and $n_1 - a_1$ taken as zero). By analogy with the case of continuous variation, the sum of squares between classes is

$$\sum_1^N \frac{a_1^2}{n_1} - \frac{(\sum a_1)^2}{\sum n_1}$$

with $N-1$ degrees of freedom.

The expected value of this sum of squares is

$$(N-1)\bar{p}(1-\bar{p}) + n_0 r \sigma_p^2$$

where r is the difference in genetic relationship between and within classes, and

$$n_0 = \sum n_1 - \frac{\sum n_1^2}{\sum n_1} - (N-1)$$

[see SNEDECOR 1946, p. 234, and KENDALL 1945, p. 123, eq. (5.29)]. The within classes component is $\bar{p}(1-\bar{p})$, which is the normal expression for the variance of a binomial population. The genetic variance then is

$$\sigma_p^2 = \frac{\left[\sum \frac{a_1^2}{n_1} - \frac{(\sum a_1)^2}{\sum n_1}\right] - (N-1)\bar{p}(1-\bar{p})}{r n_0},$$

and heritability equals

$$\frac{\sigma_p^2}{\bar{p}(1-\bar{p})} = \left[\frac{\sum \frac{a_1^2}{n_1} - \frac{(\sum a_1)^2}{\sum n_1}}{\bar{p}(1-\bar{p})} - (N-1)\right] \Big/ r n_0.$$

However, the first term in the numerator is the heterogeneity χ^2 in the $2 \times N$ table which the data form, so that heritability may be expressed as

$$\frac{\chi^2 - (N-1)}{r n_0}$$

The mean value of χ^2 for $N-1$ degrees of freedom is $N-1$. Hence the numerator of the above expression is the excess of the observed χ^2 above its expected value.

The relation of the heritability to the χ^2 statistic raises a further point. It is well-known that the χ^2 test is unreliable if the expected number in any subclass is small, the minimum per subclass being usually taken as five. COCHRAN (1936) has considered the distribution of the heterogeneity χ^2 in the case of the $2 \times N$ table with a constant number n of observations in each of the N classes. He has shown that the mean value of χ^2 is slightly greater than $N-1$, being given by

$$\chi^2_{exp} = N - 1 + \frac{1}{n}\left[1 - \frac{1}{N}\left(1 - \frac{1}{n}\right)\right].$$

The excess depends largely on n and is independent of the expected numbers in subclasses. In the cases here considered, we have approximately,

(a) in the analysis between sires within years,

$$N = 10, \qquad n = 40,$$

(b) in the analysis between dams within sires within years,

$$N = 5, \qquad n = 8.$$

For the present analysis, the samples were made fairly homogeneous as regards n by elimination of some of the observations. In case (a), all sires with n less than 12 and in case (b) all dams with n less than five were excluded. It is obvious by reference to the above formula that in the analysis between sires, n is so large that no correction need be made even for causes of death with low incidence. In the analysis between dams, however, a correction was made in all cases. It was of the order of 0.10 for each χ^2 calculated and, in the case of total mortality, for instance, reduced the estimate of heritability by about one in eight.

It is possible to give a rough estimate of the standard error of the estimates of heritability, since the method used above is similar to the determination of the intra-class correlation in the case of continuous variation. FISHER (1941) has shown that in a case similar to that discussed above and using the same symbols,

$$\sigma_t = [1 + (n-1)t](1-t)\sqrt{\frac{2}{n(n-1)(N-2)}}$$

where t denotes the intra-class correlation. Since heritability is then equal to t/r, the standard error of the estimate of heritability is given by σ_t/r.

In the data here discussed, the average size of the flock was 400–500 birds. Data for about eight breeding males, each mated to an average of five females, were used for each of the years analyzed. Owing to the considerable variation in mortality from year to year, the analyses were performed within years and the values of χ^2 and n_0 for the separate years pooled to give the final estimate.

With this flock structure, the value of r was 0.285 in the analysis between sires (each group being a mixture of full and half sisters) and 0.250 in the analysis between dams within sires (that is, for full sisters in a population of half sisters). Table 1 shows the results.

TABLE 1

Estimates of heritability

TYPE OF MORTALITY	MEAN INCIDENCE	BETWEEN SIRES		BETWEEN DAMS WITHIN SIRES HERITABILITY	JOINT ESTIMATE OF HERITABILITY
		POOLED χ^2	HERITABILITY.		
1. Total	0.416	206.39	0.0832	0.1123	0.0893±0.028
2. Lymphomatosis	0.081	165.86	0.0506	0.0315	0.0475±0.017
3. Reproductive disorders	0.234	130.68	0.0223	0.0553	0.0264±0.015
4. Other than lymphomatosis	0.335	185.25	0.0662	0.0633	0.0656±0.020

In the analysis between sires, the pooled values of n_0 and the degrees of freedom were 4358.7 and 103 in all cases. This gave a standard error of the final estimates of around 0.02–0.03. In the analysis between dams, the pooled values were 2752.3 and 414 respectively, n_0 being less than in the former case owing to the exclusion of dams with less than five progeny. This gave a standard error of the estimates of heritability of 0.04–0.05. There is no significant difference in the heritability of the different causes of death as derived from an analysis between the different sexes. In the joint estimate, the heritabilities from the two sexes were weighted according to their standard error, which resulted, on the average, in that between sires having a weight five times that between dams. An independent measure of the standard error of the joint heritability can be obtained from a consideration of the variation of the heritability between the different years. In the case of total mortality, the heritability was estimated to have a standard error of ± 0.028 from the above formula and ± 0.024 from the variation between years.

These results show a fair agreement with those of LUSH, LAMOREUX and HAZEL (1948), who found a heritability of 0.083 for total mortality compared with 0.059 from lymphomatosis deaths and 0.034 for deaths from other causes. The production index referred to by LERNER and HAZEL (1947) in their paper on genetic improvement in this flock is the average production during the laying year of all pullets housed and their estimate of individual heritability for the character was 0.045. Both they and DEMPSTER and LERNER (1947) have found that this figure is sufficiently accurate to account for the changes in the production index under selection.

The production index of the flock may be considered as made up of three constituent parts:

(i) the average production of survivors,
(ii) the average production of birds dying before the end of the year,
(iii) the relative proportion of birds falling into the above two classes, defining survival level or viability.

From the genetic viewpoint, there are two models of the situation available. The first model takes the production of surviving birds as its starting point and considers survival or death as affecting the environment in which the production record is made. The heritability of the survivors' production for this flock has been determined by LERNER and CRUDEN (1948) whose results agree closely with those of SHOFFNER (1946) on a totally unrelated flock in yielding the figure of 0.30–0.35. The present investigation has considered the heritability of the last component and has established it at around 0.09. The heritability of the production index is then lower than the heritability of either of the two components. There are three possible ways in which such a condition might arise:

(i) the heritability of the second component (production of birds dying before the end of the year) may be so low as to reduce the heritability of the production index below that of the other components;
(ii) there is a negative genetic correlation between production and viability;
(iii) non-additive genetic effects are involved.

It is impossible to discriminate adequately between the various possibilities but considerations to be presented elsewhere would suggest that the first two probably play a significant rôle.

The second genetic model, possibly slightly more logical, is to consider total production, whether the bird lives or dies, as representing the basic genotype involved. No distinction is to be made between genes controlling viability and genes controlling egg yield. Viability then becomes important as having a high environmental correlation with production and its inclusion in a selection index is desirable. It would seem likely, however, that on this scheme, there will be considerable interaction between viability and production which will cause complications in the full application of the model.

THE GENETIC CORRELATION BETWEEN MORTALITY FROM DIFFERENT CAUSES

It should be noted that cases of double classification of mortality from both lymphomatosis and reproductive disturbances do appear in the data. If there were no complications of any sort (e.g. differences in age distribution of deaths from both causes) the environmental correlation between the two types of mortality could be readily computed. This might be done by a comparison of the number of those falling in the double classification with those falling in the separate single classes. The nature of the actual data usually available precludes the possibility of such computations.

It is possible to divide the total mortality into the deaths from lymphomatosis and those from other causes. As these are mutually exclusive classes, the environmental correlations between them can be easily calculated. The following symbols must first be defined:

(i) the genotype for viability with reference to lymphomatosis, p_a with the

corresponding genotype for mortality q_a, equal to $1-p_a$. These will have variances of σ_a^2.

(ii) similar genotypes with reference to other causes, if lymphomatosis is not present, p_b and q_b with variances of σ_b^2.

If lymphomatosis is present, however, there will be cases of double classification which will be counted as due to lymphomatosis only. The apparent mortality from other causes is then reduced. We must then define:

(iii) the genotype for apparent viability with respect to other causes, when lymphomatosis is present in a population, p_c with corresponding q_c and σ_c^2. It is clear that q_c is simply related to q_a and q_b by the relationship

$$q_c = q_b - q_a q_b,$$

and total mortality q_T is then equal to $q_a + q_c$.

We can now calculate the variance of q_c and its covariance with q_a by simple algebra. We may write

$$q_a = \bar{q}_a + \delta q_a$$

where $\bar{q}_a$ is the mean of q_a and δq_a is the deviation from the mean.

Then

$$\bar{q}_c + \delta q_c = \bar{q}_b + \delta q_b - (\bar{q}_a + \delta q_a)(\bar{q}_b + \delta q_b)$$

$$= \bar{q}_b - \bar{q}_a\bar{q}_b + (1 - \bar{q}_a)\delta q_b - \bar{q}_b\delta q_a - \delta q_a\delta q_b.$$

If the variances are small compared to the mean, the last term may be ignored, giving

$$\delta q_c = \bar{p}_a\delta q_b - \bar{q}_b\delta q_a$$

and

$$\sigma_c^2 = \bar{p}_a^2\sigma_b^2 + \bar{q}_b^2\sigma_a^2 - 2\bar{p}_a\bar{q}_b r\sigma_a\sigma_b,$$

where r is the genetic correlation between p_a and p_b. Sim larly

$$\text{cov. } (p_a, p_c) = -\bar{q}_b\sigma_a^2 + \bar{p}_a r\sigma_a\sigma_b.$$

A determination of σ_a^2, σ_c^2 and cov.(q_a, q_c) will thus allow the calculation of r.

The environmental covariance can easily be calculated from the following considerations. Within a given phenotype the distribution of birds with respect to their survival or death can be arranged in a four-fold table:

		Other Causes: Alive	Other Causes: Dead
Lymphomatosis	Alive	$1-q_a-q_c$	q_c
	Dead	q_a	0

Scoring death from either lymphomatosis or other causes as zero and survivals as unity, the covariance (which here is environmental) is

$$1-q_a-q_e-(1-q_a)(1-q_e), \text{ or } -q_aq_e.$$

We can then proceed as follows in the analysis of the data. The between class covariance can be obtained as in the heritability analysis from the expression,

$$\text{between class covariance} = \sum \frac{a_1c_1}{n_1} - \frac{\sum a_1 \sum c_1}{\sum n_1}$$

where c_1 refers to birds surviving death from other causes. This, like the sum of squares between classes, consists of two terms. The first contains the environmental covariance, which can be calculated as above, and the second the genetic covariance. A comparison of the latter with σ_a^2 and σ_e^2 allows an estimation of the genetic correlation r between p_a and p_b.

The data were similarly analyzed within years but the analysis between dams was not made, since COCHRAN'S correction for χ^2 made the position uncertain. The genetic correlation between p_a and p_e was estimated as $+0.096$. A further calculation on the above lines showed that the genetic correlation between p_a and p_b was 0.26. LUSH, HAZEL and LAMOREUX (1948) found a much higher correlation (.54) between the two in their data, which they interpreted as evidence of the existence of a general genetic factor controlling resistance to death. The present correlation is positive but not particularly large, suggesting that a general factor for vigor does not play a large part in this particular flock.

Since, first y, the genotype for viability from two different causes is not additive (since $p_T=p_ap_b$) and secondly, the denominator in the equation for heritability is also not additive, the heritability of the total mortality cannot be obtained simply from the heritability of its components.

Considering the case discussed above,

$$h_a^2 = \frac{\sigma_a^2}{\bar{p}_a\bar{q}_a}$$

$$h_b^2 = \frac{\sigma_b^2}{\bar{p}_b\bar{q}_b}.$$

By putting $p_a=\bar{p}_a+\delta p_a$, etc. it can easily be shown that

$$\sigma_T^2 = \bar{p}_a^2\sigma_b^2 + \bar{p}_b^2\sigma_a^2 + 2\bar{p}_a\bar{p}_br\sigma_a\sigma_b$$

and

$$h_T^2 = \frac{\sigma_T^2}{\bar{p}_a\bar{p}_b(1-\bar{p}_a\bar{p}_b)}.$$

In the simple case, when $\bar{p}_a=\bar{p}_b$ and $\sigma_a^2=\sigma_b^2$

$$h_T^2 = \frac{2\sigma_a^2(1+r)}{1-\bar{p}_a^2}$$

$$= h_a^2(1+r)\frac{2\bar{p}_a}{1+\bar{p}_a}.$$

The heritability of total mortality depends, therefore, not only on the heritability of the components but on their mean mortality levels.

THE RELATIONSHIP OF HERITABILITY TO MORTALITY LEVEL

In the course of the analysis heritability estimates were obtained for each of the 12 years separately. There was considerable variation in the level of total mortality from year to year, as also was the case with respect to the level of the separate causes of mortality. Figures 1 and 2 show the variation of apparent

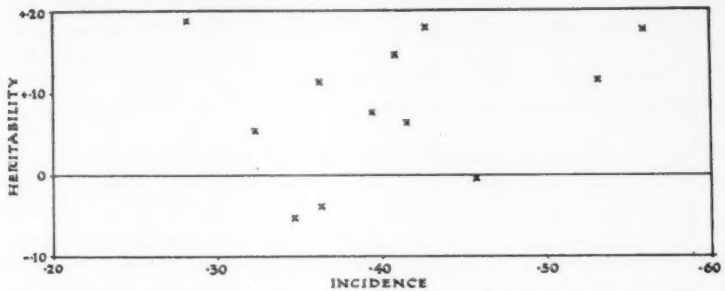

FIGURE 1.—The relation between estimated heritability and incidence of total mortality.

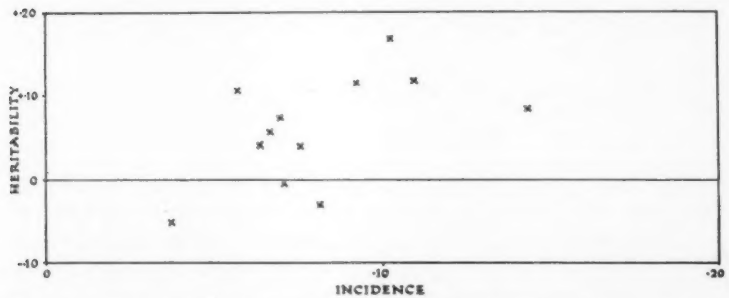

FIGURE 2.—The relation between estimated heritability and incidence of lymphomatosis.

heritability of total mortality and death from lymphomatosis in relation to the mean incidence. The values of heritability are derived from those obtained from the analysis between sires and dams, with appropriate weighting, and the negative values merely reflect values of the heterogeneity χ^2 less than the number of degrees of freedom in that year. The heritability of death due to reproductive disorders was not considered, as the mean value for it is not significantly different from zero. The variation of the points is wide but not greater than that expected theoretically. In the case of deaths from lymphomatosis, the heritability seems to be related to the average incidence of disease. In the case of total mortality, there is no apparent trend of the points, although the fitting of a regression line shows a slight positive slope. However, the range

of variation of incidence is such, in this case, that heritability would not be expected to vary greatly. In the case of death from a single specific cause, the relationship between heritability and mortality can be predicted theoretically.

The question of the transformation of the data was mentioned in the presentation of the technique of estimation of heritability. Any such transformation would be expected to affect the heritability comparatively little, since the effect is to multiply both the numerator and denominator of the heritability expression by a constant factor. The arc-sine transformation makes the denominator independent of $\bar{p}$ while the genetic variance is still dependent. On the other hand, it can be shown that in the case of mortality from one single definite cause, such as a specific infection or a poison, the probit transformation makes the genetic variance independent of $\bar{p}$ while the denominator varies in a perfectly known manner, being equal to $\bar{p}\bar{q}/\bar{z}^2$ where $\bar{z}$ is the ordinate of the normal curve at the point where the intercept is equal to $\bar{p}$. The basic concept of

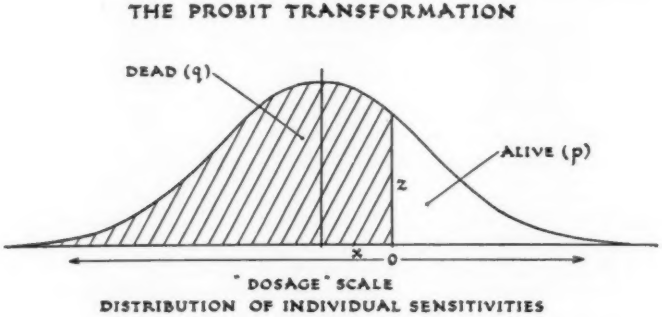

FIGURE 3.—The basic concept of the probit transformation.

the probit transformation is that the mortality is the expression of the sensitivity of the individuals in the population, the sensitivity being assumed to be normally distributed on a "dosage" scale, where the "dosage" is some measure of the level of the infection or the concentration of poison. Then all individuals whose sensitivities are below the given "dosage" will die.[2] The probit is then a measure on the dosage scale, in terms of the standard deviation within the population whose mortality is measured. Figure 3 expresses the situation pictorially.

[2] DR. EVERETT R. DEMPSTER in reviewing the manuscript has suggested a model differing from the one employed here, which may be easier to visualize but leads to the same mathematical results. He considered the genotype as the proportion of environments in which the organisms will survive. In this view the organism is seen as subjected to a distribution of environments or dosages rather than having, as in our case, its sensitivity changed by the environment and then subjected to a given dose. In our model the "probit" is derived from the dichotomy of distribution of sensitivities by the dosage; in his, from the dichotomy of the dosage distribution by the sensitivity. The theoretical implications of the two models are the same except that the concept of heritability on the dosage scale introduced by LUSH, LAMOREUX and HAZEL (see below) can not be applied to DR. DEMPSTER'S.

The probit transform of q, the mortality, is then 5+x, the 5 being introduced to avoid negative values, and x is measured in terms of the standard deviation of the group whose probit it is. In the case of the probit of a given genotype, it would be expressed in terms of the environmental variation of that genotype on the dosage scale. The whole population is then made up of the sum of all the genotypic distributions. If it be assumed that the environmental distribution on the dosage scale is independent of genotype, then on the movement of the point 0 along the scale (thereby changing the mean mortality) the mean of all the probits will be altered by a constant amount, and the variance will remain constant. That is to say, the genetic variance on the probit scale is independent of mean mortality. We can thus predict the variation of heritability with $\bar{p}$, since

$$\text{heritability} = \frac{\sigma_x^2 \cdot \bar{z}^2}{\bar{p}(1 - \bar{p})}$$

and σ_x^2 is constant (see figure 4 below). This point has essentially been made by FISHER (1947, p. 217) in noting that if the dosage-mortality relationship (corresponding to the standard deviation of the distribution mentioned above) has been determined, the toxicity of any material (and correspondingly the toxicity of a given material to an unknown genotype) can be established with an accuracy proportional to z^2/pq. The analysis presented will, however, not be applicable if σ_x^2 is large. Because of the interaction introduced by the change of scale, $\bar{p}(1-\bar{p})/\bar{z}^2$ is no longer an accurate measure of the total variance. There is, for instance, no a priori limit to σ_x^2 and therefore the heritability

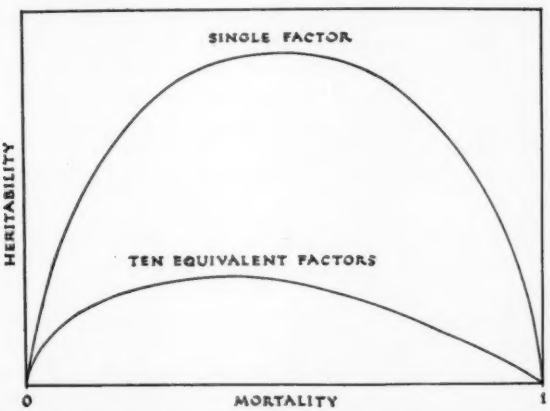

FIGURE 4.—The relationship between heritability and mortality level, in the case a) where mortality is due to a single cause to which the concept of sensitivity is applicable, where

$$h^2 = \frac{K\bar{z}^2}{\bar{p}(1-\bar{p})},$$

and in the case b) where the total mortality is compounded from that due to ten such independent equivalent causes, where h_T^2 is obtained as in the text expression.

could be greater than one. There seems to be some confusion on this point in the report of LUSH, LAMOREUX and HAZEL (1948). They have introduced the concept of "heritability on the probit scale," which, however, is not the real heritability of sensitivity but the genetic variance. The assumption implicit in their reasoning is that the probit units for the whole population and for the individual genotypes are the same, whereas in reality they are different. The probit for individual genotypes takes as its unit of measurement the environmental standard deviation on the sensitivity scale. That for the whole population takes as its unit the total phenotypic standard deviation. In terms of variance the latter is $(1+\sigma_x^2)$ times the former. Thus, the heritability on the sensitivity scale is equal to

$$\frac{\sigma_x^2}{1+\sigma_x^2},$$

rather than to σ_x^2 as considered by LUSH, LAMOREUX and HAZEL (compare WRIGHT 1934a).

The above discussion applies only to mortality from a definite single cause whose "dosage" scale has a definite biological meaning. In omnibus classifications such as "total mortality" and "other mortality" considered by LUSH, LAMOREUX and HAZEL from a similar viewpoint, the dosage scale ceases to have any definite meaning. It can only be defined in terms of the mortality level itself, which is made up of any number of different factors with different heritabilities, and may be so made up in an infinite number of possible ways. The situation is considerably removed from the case of the single disease where the change in mortality can be described as a point moving along a sensitivity scale. We can then only speak of the average heritability at a given level, the average being taken over all possible components of the factors which combine to give that total level. It is obvious that if we have two separate factors with very different heritabilities, the heritability of the joint mortality at the level of 50 percent will differ greatly depending whether, for instance, the highly heritable factor has a level of 40 percent and the other a level of 17 percent, or *vice versa*.

The problem may be considered by use of the method presented in the last section for calculating the total heritability of two factors. The method can be extended to any number of factors (A, B, C, etc.) to give the general result

$$h_T^2 = \frac{p_T}{1-p_T}\left(\sum \frac{K_a z_a^2}{p_a^2} + 2\sum r_{ab}\frac{\sqrt{K_a K_b}\, z_a z_b}{p_a p_b}\right)$$

where K_a is the genetic variance of mortality due to A on the probit scale, and r_{ab} the genetic correlation of mortality due to A and B, or A and C, and so forth.

This formula has been applied to two cases as follows:

(i) the total mortality is made up of several equivalent uncorrelated factors. Figure 4 shows the heritability of total mortality made up of ten factors, the upper curve being the heritability of one such factor alone. It will be

seen that the heritability of the compound mortality is always less than that of the single factor at the same level, the relative difference being greater at high mortalities. At 50 percent mortality it seems to be roughly true that the heritability of mortality due to m equivalent factors is $1/\sqrt{m}$ times the heritability of one such factor at the same level. In addition, the curve is no longer symmetrical but is pushed over towards the lower mortality levels. It is obvious that the variation with mortality level is quite different from that of the single factor.

(ii) the total mortality is made up of two uncorrelated factors, the variation from season to season being due mainly to one factor only. Figure 5 shows the variations of heritability with total mortality when two factors, A and B, are involved, such that:

(a) A has a heritability of 0.10 at its mean level of 10 percent with a standard deviation of mean level from season to season of 0.04.

(b) B has a heritability of 0.02 at its mean level of 33.3 percent with a standard deviation of mean level from season to season of 0.13.

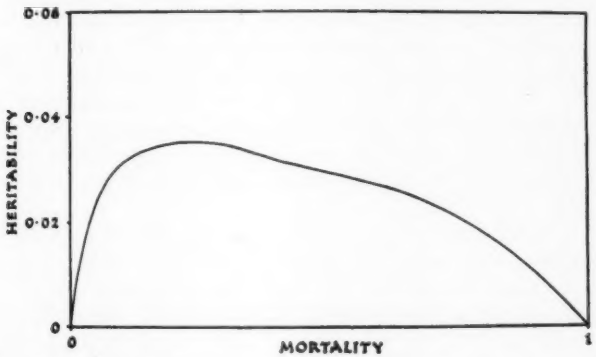

FIGURE 5.—The relationship between heritability and compound mortality from two causes for the conditions specified in the text.

Here the curve is very skew with a maximum around 20 percent mortality. In fact, the heritability varies little over the range 0.10–0.50. By varying the different constants controlling A and B, the maximum could be shifted to any desired level. This case, in which most of the yearly variation is contributed by a component, whose heritability is relatively low, will be the one departing most from the theoretical curve.

It would seem likely from these two instances that in the majority of practical cases, although it will be impossible to predict the exact variation of heritability of total mortality with incidence, the maximum will probably be in the region between 25 percent and 60 percent mortality.

The genetic variance on the probit scale of any two independent factors would not be expected to be additive, due to the high interaction between the

TABLE 2

Probit values in relation to the level of mortality from two causes

MORTALITY DUE TO	LEVEL	PROBIT	ADDITIONAL DUE TO A
B	10%	3.72	
A+B	19%	4.12	0.40
B	50%	5.00	
A+B	55%	5.12	0.12

two probits as is shown by table 2, which illustrates the effect of adding factor A at 10 percent mortality to factor B at 10 percent and 50 percent. The difficulties of the use of the probit transformation in the case of deaths from independent causes has been fully discussed by FINNEY (1947 p. 136).

SUMMARY

1. A method is presented for the determination of the heritability of all-or-none characters, with special reference to mortality. It can be extended to cover the genetic correlation between mortality from two different causes.

2. Analysis of the mortality records of the production flock of the UNIVERSITY OF CALIFORNIA gave a heritability of 0.089 for total mortality, 0.026 for deaths from reproductive disorders, 0.048 for deaths from lymphomatosis and 0.066 for deaths from other causes than lymphomatosis. The genetic correlation between mortality from lymphomatosis and from other causes was found to be +0.26.

3. The heritability varied considerably with the mean level of mortality. A theoretical expression for this variation is derived for the case of mortality from a single specific cause.

4. The relationship of the heritability of total mortality to incidence is, however, more complex and formulae are presented giving the heritability of total mortality in terms of the levels and heritabilities of its components. These are applied to two general cases, showing that the simple laws pertaining to deaths from single causes do not hold for aggregates of causes. The deviations are likely to be greatest when the variation in mean level from season to season is mainly due to a factor with relatively low heritability.

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INHERITANCE OF MUTATIONAL CHANGES INDUCED BY RADIATION IN PARAMECIUM AURELIA

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IN a previous paper KIMBALL (1949) has described the induction by radiation of changes in vigor in *Paramecium aurelia* which first expressed themselves in the exautogamous progeny of rayed animals. In that paper, it was assumed that the changes were due to mutations in the micronucleus. This paper will present the genetic evidence which bears on this assumption.

The radiation studies suggested that most of the reduction in vigor after autogamy was due to the combined action of a number of mutant genes with individual effects too small to be detected rather than to genes with large effects. Thus, the phenomena of inheritance observed should be those characteristic of multiple gene inheritance.

METHODS

For this work stock 90 of variety 1 was used. Four experiments were carried out. In the first two, beta radiation was given by means of the phosphorus plaque technique previously described. In experiment 1 approximately 13,000 rep were given; in experiment 2, approximately 6,500. The dosage rate was about 200 rep per minute. The irradiation in experiments 3 and 4 was by X-rays from a Maximar model machine operated at 250 kv and 15 ma. There was an inherent filtration of 3 mm of aluminum but no added filtration. Measurements were made immediately before exposure with a Victoreen chamber placed in the same position as the exposure chamber. The animals in 0.5 cc of culture fluid were put in a cavity 1.8 cm in diameter drilled in a small lucite block, and the cavity was covered with cellophane tape. The block was placed under the tube so that the animals were about 46 cm from the target. In experiment 3, a dose of 6,600 r was given; in experiment 4, 7000 r. The dosage rate was about 245 r per minute.

After irradiation, single rayed animals were isolated and allowed to divide. Every day a single descendent of each rayed animal was isolated to carry on the line of descent. A few days after irradiation the descendants of animals left over after the first or second isolation were mixed with unirradiated animals of the opposite mating type and conjugant pairs isolated from the mixture. When the conjugants had separated, the two members of each pair were put in separate containers. One product of the first or second division of each

[1] Valuable assistance was given by NENITA T. GAITHER and MARGARET S. GRIESS.

[2] This document is based on work performed under Contract No. W-7405-eng-26 for the ATOMIC ENERGY COMMISSION at OAK RIDGE NATIONAL LABORATORY.

exconjugant was saved and the rest discarded to prevent mixture of different caryonides and the reconjugation which such mixture would allow. The animals which were saved were left in a limited quantity of culture fluid for four days and then observed for total growth. All those which failed to produce a maximum and starved population in this period were classified as of reduced vigor. This is the same test for vigor previously described in some detail by KIMBALL (1949). The group of exconjugants obtained in this way will be called the BC1 generation.

Meanwhile the line of descent from the rayed animals was continued by daily isolation and about a week after irradiation the left-over animals from each line were found to be in autogamy. Twenty-five autogamous animals were isolated from each rayed line and likewise subjected to the four-day total growth test. This generation will be called the A1.

The exconjugants of the BC1 generation likewise were continued by the daily isolation technique. About a week after conjugation in experiments 1 and 2, some of the progeny from several of the clones were caused to conjugate again with unirradiated animals to yield a BC2 generation. In all four experiments, two to three weeks after conjugation, autogamy was found to be occurring in the left-overs from daily transfer, and 25 autogamous animals were isolated from each BC1 clone. This will be called the A2 generation. In experiment 1, BC3 and A3 generations were obtained in the same manner from the BC2 clones. In all four experiments, unirradiated animals from the same clone as the original irradiated ones were carried through the same breeding procedure to serve as controls.

GENETIC EXPECTATIONS

According to the conclusions of KIMBALL (1949), animals given the doses of radiation employed here are probably heterozygous for a number of mutant genes affecting vigor. When such heterozygotes are crossed to unrayed animals the exconjugants will be, on the average, heterozygous for a number of mutant genes but will rarely, if ever, be homozygous for any of them. Different exconjugant pairs from the same rayed animal will be heterozygous for different combinations of mutants as a result of segregation during gamete nucleus formation in the rayed member of the pair. However, the two members of a pair should be of identical genotype after conjugation. The average number of mutant genes per micronucleus in the BC1 generation should be half that in the original rayed animals. Exautogamous animals should be completely homozygous and, if derived from heterozygous animals, should frequently be homozygous for some of the mutant genes. Different exautogamous animals from the same heterozygote should be homozygous for different combinations of mutants as a result of segregation during gamete nucleus formation. For the details of the genetic and cytological information upon which these expectations are based, SONNEBORN (1947) may be consulted.

In testing the backcross generations for agreement with these expectations, the phenotype of the backcross clones themselves, as expressed in four-day total growth, might be used. However, this method would only detect mutants

with some dominant effect. A more satisfactory method is to obtain exautogamous progeny from each backcross clone. If a large number of autogamous progeny were obtained, all possible homozygous combinations of the mutant genes in the backcross clone should appear; and the fraction of exautogamous clones of reduced vigor should be characteristic for the genotype of the backcross clone. In general, the more mutant genes, the larger this fraction would be. In actual practice only 25 autogamous progeny were obtained from each clone so that this measure is expected to be subject to considerable sampling error.

PHENOTYPE OF THE BACKCROSS CLONES

The data on the phenotype of the backcross clones as expressed in four day total growth are given in table 1. Each clone was classified as of normal vigor (+) or of reduced vigor (−). Thus, there were three classes of pairs depending upon the phenotype of the two members.

The BC1 of experiments 1, 2, and 3 and the BC2 of experiment 2 do not differ significantly from each other, as shown by a chi-square for heterogeneity

TABLE 1

Data on exconjugant generations

GENERATION	EXPERIMENT	NUMBER OF ORIGINAL RAYED OR CONTROL ANIMALS	NUMBER OF CLONES FROM PREVIOUS GENERATION FROM WHICH DERIVED	NUMBER OF PAIRS			
				++	+−	−−	TOTAL
BC1	1	5	—	112	4	0	116
	2	9	—	206	16	6	228
	3	30	—	107	8	4	119
	4	13	—	98	23	9	130
	Total	57	—	523	51	19	593
BC2	1	1	6	45	17	28	90
	2	3	60	541	37	6	584
BC3	1	1	34	407	20	60	487
BC1 Control	1	1	—	30	0	0	30
	2	6	—	140	15	5	160
	3	10	—	37	3	0	40
	4	5	—	47	3	0	50
BC2 Control	1	1	2	20	0	0	20
	2	1	20	162	6	0	168
BC3 Control	1	1	10	147	3	0	150
Total Control	—	22	—	583	30	5	618

of 9.3 for six degrees of freedom, nor does their total differ significantly from the total for all the controls as shown by a chi-square for heterogeneity of 4.3 for two degrees of freedom. The BC1 of experiment 4 differs significantly from the total controls as shown by a chi-square of 50 for two degrees of freedom. The BC2 and BC3 of experiment 1 also differ significantly from the controls as shown by high values of chi-square. The later generations of experiment 1 show other peculiarities, and a detailed discussion will be reserved for another section. Possibly, the divergence of the results of experiment 4 from the others is due to the chance occurrence of more dominant mutations in it than the others, but the number of discordant pairs makes difficulties for this interpretation.

Except for experiment 4, there seems to be no evidence for dominant effects in BC1. This is so despite the fact that all 57 rayed animals from which this generation was derived gave evidence in their A1 progeny of effects of the radiation. Many of them produced more than 50 percent clones of reduced vigor, and the smallest percentage was 24 percent as compared to controls which rarely exceed 16 percent. It is possible that dominant changes are sufficiently less frequent than recessives with small effects and that the relatively small group of rayed animals so far studied is inadequate to demonstrate them clearly.

The occurrence of discordant pairs is not expected on simple genetic theory. Since such pairs also occur in the controls, part at least have no connection with the effects of radiation. The numerous discordant pairs in experiment 4 cannot be accounted for in this way. It is possible that some may have been the result of cytogamy. This process, which was not distinguished from true conjugation in the present work, is essentially autogamy in the two members of the pair. The member of rayed ancestry of a cytogamous pair would be just as likely to be of reduced vigor as any autogamous animal from the same rayed animal. The member of unrayed ancestry would be just as unlikely to be of reduced vigor as the autogamous descendants of the corresponding unrayed animal. It should be noted that in most cases later generations were obtained from BC1 pairs, both members of which were normal. Thus, the animals of these generations were descended probably from few, if any, cytogamous pairs. Most pairs from which later generations were obtained were probably true conjugants.

THE EXAUTOGAMOUS PROGENY OF BC1 CLONES

As has already been pointed out, the A2 generation should furnish a measure of the genotype of the BC1 clone from which it was derived. An A2 generation was obtained from both members of 124 BC1 pairs and from one member only of 23 other pairs. From each member about 25 autogamous animals were obtained. An A2 generation was not always obtained from the same numbe of BC1 pairs for different rayed animals. For purposes of a statistical analysis to be given below, it was desirable to have a body of data in which an A2 was obtained from the same number of BC1 pairs for each rayed animal. Therefore, for each rayed animal for which at least two pairs were available two were

TABLE 2

Fraction of A2 clones of reduced vigor

EXPERIMENT	RAYED ANIMAL	BC1 PAIR	MEMBER OF PAIR a	MEMBER OF PAIR b
1	1	1	0.96	0.96
		2	0.84	1.00
	2	1	0.96	1.00
		2	1.00	1.00
	3	1	0.04	0.08
		2	0.28	0.00
2	1	1	0.76	0.00
		2	0.20	0.00
	2	1	0.04	0.12
		2	0.04	0.16
	3	1	0.16	0.24
		2	0.00	0.00
3	1	1	0.56	0.32
		2	0.00	0.24
	2	1	0.56	0.40
		2	0.76	0.28
	3	1	0.32	0.44
		2	0.96	0.68
	4	1	0.36	0.56
		2	0.64	0.80
	5	1	0.48	0.40
		2	0.56	0.84
	6	1	0.96	0.88
		2	0.16	0.20
	7	1	0.80	0.20
		2	0.88	0.96
	8	1	0.32	0.44
		2	0.56	0.20
	9	1	0.76	0.64
		2	0.52	0.52
	10	1	0.60	0.44
		2	0.16	0.44
	11	1	0.60	0.44
		2	0.60	1.00
	12	1	0.52	0.96
		2	0.32	0.28
	13	1	0.48	0.68
		2	0.12	0.12
	14	1	0.44	0.44
		2	0.28	0.48
	15	1	0.48	0.96
		2	0.68	0.80
	16	1	0.00	0.00
		2	0.08	0.00
	17	1	0.48	0.40
		2	0.28	0.52
	18	1	0.16	0.04
		2	1.00	0.52
4	1	1	0.96	1.00
		2	0.52	0.84
	2	1	0.28	0.32
		2	0.68	0.72
	3	1	0.68	0.72
		2	0.88	0.60
	4	1	0.88	0.80
		2	0.80	0.16
	5	1	0.20	0.16
		2	0.00	0.00
	6	1	1.00	0.92
		2	1.00	0.68
	7	1	0.36	0.44
		2	0.28	0.64
	8	1	0.08	0.56
		2	0.00	0.00
	9	1	0.32	0.36
		2	0.92	0.68
	10	1	0.96	1.00
		2	0.88	0.96
	11	1	0.80	1.00
		2	1.00	0.44
	12	1	0.20	0.80
		2	0.80	1.00
	13	1	0.00	0.00
		2	0.12	0.08

chosen at random using SNEDECOR'S (1946) table of random numbers. This random sample of the total data is presented in table 2. It consists of the A2 generation from 74 pairs derived from 37 rayed animals from all four experiments. The body of the table shows the fraction of A2 clones of reduced vigor.

The two members of the BC1 pairs are labeled "a" and "b" arbitrarily since it was not known which came from the rayed and which from the unrayed member. Control data have been omitted to save space. Suffice it to say that only occasionally was the fraction of reduced vigor in the controls higher than 0.16 (four clones out of 25) though a few very high fractions were found, especially in experiment 4. An examination of the table clearly demonstrates that the effect of the radiation is transmitted to both members of the pairs for, in many cases, both members produced much higher fractions than were characteristic of the controls.

TABLE 3

Analysis of variance of the A2 data in table 2

	MEAN SQUARE	DEGREES OF FREEDOM
(1) Variance attributable to differences between the progeny of different rayed animals	1823	36
(2) Variance attributable to differences between the pairs from the same rayed animal	477	37
(3) Variance attributable to differences between members of the same pair	203	74

Variance ratio of (2)/(3) = 2.35
Probability = less than 0.01

The expectation that the two members of a pair should be of identical genotype and so alike in the fraction of exautogamous clones of reduced vigor which they produce is not met with any exactness by the data in table 2. At least two factors might be expected to cause departures from identity. The first is deviation due to sampling error which, with samples of 25, is expected to be high. The second is spontaneous mutation. The exconjugants had to multiply vegetatively for two to three weeks before exautogamous clones could be obtained. During this time, spontaneous mutation could occur and destroy the original identity of genotype.

Even if spontaneous mutation were relatively frequent, the two members of a pair should tend to be more alike than different pairs from the same rayed animal since different pairs would often be of different genotype immediately after conjugation and spontaneous mutation could only add to a difference already existing. To see if this were so, an analysis of variance was carried out on the data of table 2. The fractions were first converted to angles using the table of this transformation in SNEDECOR (1946). The sums of squares attributable to differences between the progeny of different rayed animals, to differences between pairs from the same rayed animal, and to differences between members of the same pair were found and divided by their degrees of freedom to give the mean squares shown in table 3. As would be expected, the

mean square for differences between rayed animals is considerably greater than the other two. The ratio of the mean square for differences between pairs from the same rayed animal to that for differences between members of the same pair is 2.35. According to the table of the variance ratio given by SNEDECOR (1946), a value as great or greater than this would have less than one chance in a hundred of occurring if the two mean squares were really the same. It can be concluded that the two members of a pair are more alike than are different pairs from the same rayed animal.

The final expectation that can be tested with the exautogamous generations is that the mean number of mutant genes per micronucleus should be halved by backcrossing to unrayed animals. KIMBALL (1949) gives an equation by which the mean number of mutant genes can be calculated from the average fraction of their autogamous progeny of reduced vigor. The equation in its most general form is:

$$F = 1 - e^{-m/2}[c_0 + c_1(m/2) + (c_2/2!)(m/2)^2 + (c_3/3!)(m/2)^3 + \cdots]$$

where F is the average fraction of exautogamous clones of reduced vigor; m is the mean number of mutant genes per micronucleus; and c_0, c_1, c_2, etc. are constants representing the probability that clones with respectively none,one, two, etc. homozygous mutant genes will be of normal vigor. The dosage data led to the conclusion that c_0, c_1, c_2, c_3 are unity and c_5, c_6, etc. are zero, with a question as to what value to assign c_4. For present purposes, it will be assumed to be zero, a value which gives a good fit to the dosage data. Using this equation, m can be calculated for the rayed animals from the A1 data; and for the BC1, from the A2 data.

Experiment 1 is entirely unsuited for such calculations since the A1 genera-

TABLE 4

The mean number of mutations per micronucleus in the rayed and BC1 animals as calculated from the A1 and A2 data

EXPERIMENT AND TREATMENT	NUMBER OF ANIMALS	GENERATION	TOTAL CLONES	FRACTION OF CLONES OF REDUCED VIGOR	MEAN NUMBER OF MUTANT GENES	RAYED MINUS CONTROL	RATIO A1/A2
2—rayed	3	A1	75	0.753	10.3	10.3	3.7
	3	A2	1469	0.127	4.0	2.8	—
2—control	6	A1	150	0.000	0.0	—	—
	1	A2	500	0.004	1.2	—	—
3—rayed	30	A1	749	0.826	11.5	9.6	2.3
	30	A2	2750	0.483	6.8	4.2	—
3—control	10	A1	250	0.016	1.9	—	—
	10	A2	950	0.043	2.6	—	—
4—rayed	13	A1	325	0.698	9.5	6.8	1.7
	13	A2	1300	0.582	8.2	4.0	—
4—control	10	A1	250	0.050	2.7	—	—
	5	A2	401	0.161	4.2	—	—

tion had so high an average fraction of reduced vigor (0.96) that m for the rayed animals cannot be calculated with any confidence. This is true because, as F approaches unity, a slight change in its value makes a great change in the calculated value of m. Experiment 2 is not especially satisfactory because of the low number (three) of rayed animals used. The values of the constants in the equation are average probabilities for many genes. With a small sample of mutant genes, such as would be obtained from only three rayed animals, the values might be entirely different. However, calculations for this experiment have been included since the values of F fall within the usable range. Experiments 3 and 4 are both reasonably suitable.

The calculations are set forth in table 4. Since the average fraction of reduced vigor in the controls was somewhat variable, was uniformly greater for the A2 than for the A1, and was unusually high in the A2 of experiment 4, it seemed necessary to introduce a correction for it. There are several ways in which the correction could be made depending upon the cause of reduced vigor in the controls. It seemed best to adopt a uniform hypothesis for the cause of reduced vigor and assume that, in the controls, it was due to spontaneous mutation. This is probably not entirely true; but, in the absence of data as to the nature and relative frequency of other causes, this appears to be the best approach. For this assumption, the proper correction would be the subtraction of the mean number of mutant genes calculated from a given control group from that for the corresponding rayed group. The remainder would be the mean number of mutant genes derived from those induced by the radiation.

The last column of table 4 shows the ratio between the mean number of mutant genes in the rayed animals and in the BC1. For experiments 3 and 4, the value is quite close to the expected value of two, particularly when the number of approximations involved in the calculations is taken into account. The value for experiment 2 is not so close to expectation, but it has already been pointed out that this experiment is not particularly satisfactory for such calculations.

ANOMALOUS INHERITANCE IN EXPERIMENT 1

It has already been mentioned that the BC2 and BC3 of experiment 1 show some unexpected features. The BC1 of this experiment did not include significantly more clones of reduced vigor than did the controls, but many such clones appeared in the BC2 and BC3. That the difference is real is shown by the contingency chi-square for agreement with the total controls. For the BC2, it was 182 and for the BC3, 59. Both values for two degrees of freedom have a negligible chance of occurring as a result of chance deviations. It can also be shown that there is a distinct tendency for the two members of a pair to be alike. The chi-squares for agreement with a random distribution of reduced vigor to the two members of a pair are 33 for the BC2 and 338 for the BC3. Both chi-squares indicate a highly significant departure from a random distribution, obviously in the direction of too few $+-$pairs. Thus, whatever is involved tends to affect the unrayed as well as the rayed member of the pair within a four day period after conjugation. The first explanation that comes

to mind is that dominant mutations are concerned; but if this were so, it is difficult to understand how they passed through the BC1 generation without expressing themselves.

A more detailed examination of the data reveals further peculiarities which are brought out in table 5. It was found that almost all the BC2 and BC3 pairs in which both members were of reduced vigor came from a few BC1 and BC2 clones. Moreover, in all cases in which both members of BC1 or BC2 pairs were followed, if one member produced much reduced vigor at the next conjugation, the other did not. The member which produced many clones of reduced vigor on conjugation with unrayed animals produced at autogamy many

TABLE 5

Anomalous inheritance in experiment 1

PAIR	BC1 ORIGIN	MEMBER OF PAIR											
		a						b					
		BC2 OR BC3			A2 OR A3			BC2 OR BC3			A2 OR A3		
		++	+−	−−	+	r	d	++	+−	−−	+	r	d
BC1-39	—	10	2	3	0	25	0	3	4	8	0	6	19
BC1-40	—	15	0	0	5	18	2	2	5	8	0	10	15
BC1-41	—	1	5	8	1	8	16	14	1	0	0	18	7
BC2-32	41b	not obtained			not obtained			1	3	1	0	14	11
BC2-38	41b	11	2	0	5	19	1	1	1	13	0	6	19
BC2-45	41b	not obtained			not obtained			2	1	11	0	5	20
BC2-76	40a	0	4	10	0	7	18	13	1	0	not obtained		
BC2-88	40a	14	5	2	0	25	0	1	2	19	2	6	17

+=normal vigor, −=reduced vigor, d=died within four days, r=reduced vigor but survived four days or more.

clones which failed to survive the four day period. The member which produced few clones of reduced vigor at the next conjugation produced few clones at autogamy which failed to survive the four day period though it produced many which were of reduced vigor. Finally, it should be noted that the BC2 clones which produced much reduced vigor at conjugation and death at autogamy were derived from the members of the BC1 pairs which did not show this behavior. The other member of these same pairs did show it. Apparently, the anomalous behavior can be transmitted through clones which do not exhibit it. No adequate explanation for these phenomena can be offered though it is clear that some kind of inheritance is involved. It is to be noted that table 5 includes only those pairs which showed the anomalous behavior. This includes all three BC1 pairs from which later generations were obtained but only a part of the BC2 pairs from which a BC3 and A3 were derived.

HOMOZYGOUS CLONES OF REDUCED VIGOR

A number of viable exautogamous clones from the backcross progeny of rayed animals have been obtained, some of which had distinctly lower fission rates than the controls. Such clones were presumably homozygous for one or more mutants with a relatively mild total effect upon vigor. Crosses with unrayed animals have shown that the low fission rate can be transmitted through both members of conjugant pairs, but the data are still too limited to warrant detailed presentation.

A most noticeable feature of these homozygotes was the great variability within the clone. When a number of animals were isolated from such a clone, the descendants of some had normal fission rate, or nearly so. Those of others had a distinctly lower rate, while still others failed to survive at all. When animals were isolated from the apparently normal variants, this same variability reappeared. This great variability makes detailed investigations of inheritance with this material very difficult.

DISCUSSION

SONNEBORN'S (1947) review of the genetics of *Paramecium aurelia* has brought out the diversity of the types of inheritance and has shown that a number of persistent diversities in this organism are not due to genic differences. This emphasizes the need for caution in interpreting persistent diversities in this organism as having a genic basis. Therefore, alternatives to the gene mutation hypothesis presented in this paper will be examined in some detail.

The first alternative to be considered is some form of cytoplasmic inheritance. The fact that the changes in vigor first appear after autogamy does not itself rule out this possibility since sudden changes in cytoplasmically determined characters are known to occur after this process. Transmission to both members of pairs between irradiated and unirradiated animals is not to be expected with cytoplasmic inheritance if no exchange of cytoplasm occurs between conjugants. However, evidence that only small amounts of cytoplasm, if any, are exchanged has been obtained so far only for the B group of varieties and is not available for the A group to which stock 90 belongs. As a matter of fact, evidence for cytoplasmic inheritance at conjugation has not been found so far in the A group. With cytoplasmic inheritance, the two members of a pair would not necessarily be more alike than members of different pairs from the same rayed animal. However, if cytoplasmic exchange did occur regularly and certain other conditions were met, such a result would be possible. With cytoplasmic inheritance, there is no obvious reason why m, as calculated in this paper, should be halved though again it might be possible with certain assumptions about the nature of the cytoplasmic inheritance to account for this result. Finally, the small amount of reduced vigor in the BC1, as compared with the large amounts in the A1 and A2, is not consistent with our present knowledge of cytoplasmic inheritance. In all cases so far known in which a cytoplasmically determined variation can express itself after autogamy, it can likewise express itself after conjugation. We can conclude that one feature of the data is at

variance with what is known of cytoplasmic inheritance and that the others can be made to fit such an interpretation only with special assumptions. On the other hand, all these features are expected if genic inheritance is involved.

There is one point concerning cytoplasmic inheritance that is worth emphasizing. The present experiments were not especially designed for its detection or for the production of detectable changes in cytoplasmic mechanisms of inheritance. The fact that no effects were detected as long as the animals multiplied vegetatively suggests that higher doses of radiation or more sensitive methods of detection or both will be necessary before effects on cytoplasmic components of inheritance are found. This paper gives no evidence against the occurrence of cytoplasmic inheritance in variety 1, but merely shows that it was not a major factor in most of the phenomena considered.

A type of inheritance that is possibly nongenic and is known to occur in stock 90 is caryonidal inheritance which SONNEBORN (1947) discusses in some detail. It is possible that some of the mutant genes are analogous to the gene already known in variety 1 which makes it possible for the two caryonides of a clone to be of different mating type. In the present case this would mean that one caryonide of a clone might be of reduced vigor while the other was normal, the mutant gene merely determining that these two alternatives are possible. Such a situation would not change the major conclusions though it might affect the calculation of the mean number of mutant genes from the average fraction of reduced vigor.

The question of whether gross changes in the nuclear apparatus may be of primary importance in determining reduction in vigor after autogamy needs to be considered. For example, it would be conceivable that radiation destroys or prevents the division of micronuclei thus leading to the production of amicronucleate animals. Such animals would probably be viable as long as they multiplied vegetatively, but autogamy in them might lead to death and reduced vigor. Overlooking the difficulties in fitting the detailed findings to such an hypothesis, it can be said that an amicronucleate condition is certainly not common in the progeny of rayed animals. The vegetative descendants of a number of rayed animals have been found to have micronuclei on microscopic examination. In the course of staining cultures from several hundred rayed animals as part of the routine procedure for obtaining autogamous animals, typical macronuclear anlagen have been seen repeatedly. There is no known mechanism by which such anlagen could be formed in amicronucleate lines of descent.

It might be possible that macronuclear regeneration occurs with a high frequency in the autogamous progeny of rayed animals. Since this process leads to changes which might be classified as reduction in vigor, such an occurrence might be used to explain reduced vigor in the A1. How it could be used to explain the various phenomena of inheritance, especially the lack of reduced vigor in the BC1, is not clear. In the routine examination of stained samples of cultures derived from rayed animals, a number have been found to be in late stages of autogamy at which time the typical appearance of macronuclear regeneration should have been found if it were at all common. Occasional cases

have been seen but the impression has been gained that it is no more frequent than in the controls.

In conclusion, it can be said that most of the evidence is consistent with genic inheritance and that the major expectations of this hypothesis which have been tested have been met. Nongenic inheritance, unless quite different from any now known, does not explain the data and is sometimes at variance with them. On the other hand, the anomalous inheritance in experiment 1 emphasizes the possibility that other types of inheritance may play a role, though probably a secondary one.

KIMBALL (1949) used the working hypothesis that reduced vigor after autogamy in irradiated animals was due to mutational changes in the micronucleus. This hypothesis is now confirmed. The inheritance data give little basis for a decision as to the kinds of mutational change involved. However, the data of the previous paper make it probable that gene mutations or small deficiencies with minor effects on vigor are the most important contributors to the total effect while gene mutations with major effects on vigor and two-hit chromosomal aberrations play, at most, a minor role. The inheritance studies confirm this conclusion in that the formula for the mean number of mutant genes per micronucleus developed on this basis gives the expected results when applied to the A1 and A2 data. Although the phrase *gene mutation* has been used throughout this paper, nothing in the data excludes from consideration small one-hit deletions with minor effects on vigor when homozygous.

SUMMARY

1. A study has been carried out in *Paramecium aurelia* of the inheritance of effects of radiation which first expressed themselves as reductions in vigor of some of the exautogamous progeny of rayed animals.

2. The technique used in investigating the inheritance was to cross the rayed animals to unrayed ones by conjugation. In some cases, the products of the cross were again crossed to unrayed animals. The genotypes of the products of the crosses were tested by sending their descendants through autogamy to bring about complete homozygosis.

3. The clones from both members of the conjugant pairs were in the majority of the cases of normal vigor.

4. The exautogamous progeny from both members of the pair often included a high proportion of clones of reduced vigor indicating that the effect was transmitted to both members.

5. There was clear evidence that the two members of a pair produced exautogamous generations which were more alike on the average than were those from different pairs from the same rayed animal.

6. Using an equation developed by KIMBALL (1949) on the basis of data from dosage experiments, it was possible to calculate the average number of mutations per micronucleus in the rayed animals and in the animals resulting from the cross of rayed to unrayed. The average number in the former was approximately twice that in the latter as would be expected if gene mutations were involved.

7. These facts taken together are in accord with an interpretation of genic inheritance. They can be made to fit an interpretation of non-genic inheritance only with special assumptions if at all.

8. In one experiment, an anomalous type of inheritance was found for which no adequate explanation can be offered.

9. Viable homozygous clones of lowered fission rate have been obtained by autogamy in the descendants of rayed animals. The lowered fission rate can be shown to be transmitted through both members of conjugant pairs between the low fission rate clones and normal animals.

10. A noticeable feature of such viable but low fission rate clones is the great intraclonal variability in respect to fission rate and even viability itself.

11. It can be concluded that the results of the crosses have confirmed the assumption made by KIMBALL (1949) that reduced vigor in the exautogamous progeny of rayed animals is an expression of micronuclear mutations induced by the radiation.

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MODIFICATION OF THE FREQUENCY OF CHROMOSOMAL REARRANGEMENTS INDUCED BY X-RAYS IN DROSOPHILA. IV. POSTTREATMENT WITH NEAR INFRARED RADIATION[1]

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PREVIOUS studies showed that exposure of spermatozoa of *Drosophila melanogaster* to near infrared radiation before treatment with X-rays effected a marked increase in the frequency of viable types of chromosomal rearrangement as compared with the frequency in controls receiving only the X-rays. On the other hand, exposure of males to near infrared radiation after treatment with X-rays did not significantly modify the frequency of induced rearrangements among the chromosomes of those spermatozoa that were utilized in insemination during the first few days following termination of the X-ray treatment. If, however, a period of ten days or longer intervened between the X-ray treatment and copulation, the spermatozoa derived from males receiving near infrared radiation during all or part of that period yielded a smaller proportion of viable rearrangements than the X-rayed controls; and this result was attributed to an accelerating effect of the near infrared radiation on processes that make available for transfer in copulation sperm that is not mature at the time of X-ray treatment (KAUFMANN and GAY 1945; KAUFMANN, HOLLAENDER, and GAY 1946). As was indicated in these reports, any effect of the treatment on the progress of spermatogenesis could be obviated, and more conclusive data concerning the effect of posttreatment on chromosome recombination could be obtained, if the near infrared radiation were administered after transfer of the X-rayed spermatozoa to the seminal receptacles of the female. In preliminary experiments of this type, oviposition proceeded with such celerity during the period of exposure to the near infrared radiation—24 to 48 hours—that at its termination the supply of treated sperm was largely exhausted, and few fertilized eggs were deposited subsequently. These experiments have now been extended by treating larger numbers of females, under conditions less conducive to oviposition. Among the progeny obtained from eggs deposited after such combination treatment, the frequency of chromosomal rearrangements was not significantly different from that in the X-ray controls. These results indicate, therefore, that posttreatment with near infrared radiation is not effective, as is pre-treatment, in modifying the frequency of chromosomal rearrangements induced by X-radiation of mature spermatozoa of *D. melanogaster*.

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EXPERIMENTAL PROCEDURE

Males of the Swedish-b[6] stock were selected, three to five days after their emergence, and exposed to about 5000 roentgens of X-rays, using a Coolidge tube operating at about 85 kilovolts and 5 milliamperes. At the termination of the treatment the males were mated with virgin females of the same stock; after 24 hours the males were discarded and the females divided into two groups of equal numbers. One of these, which served as a control, was stored at a temperature of 25°C. The other was exposed to the near infrared radiation for a period of 24 hours under conditions similar to those described previously (KAUFMANN et al. 1946). Oviposition was retarded by maintaining the flies—controls as well as infrared-treated—during the period of mating and the ensuing 24 hours on a diet that consisted exclusively of a five percent sucrose solution (KALMUS 1942). Frequency of rearrangements was determined by cytological analysis of acetic-orcein-stained preparations of salivary-gland chromosomes of the F_1 female larvae.

RESULTS

Quantitative aspects of the analysis are presented in the first two lines of table 1. No significant difference, with respect to frequency or complexity of

TABLE 1

Effect of posttreatment with near infrared radiation

TREATMENT (X-RAY IN r, NEAR INFRARED IN HOURS)	TOTAL SPERMS TESTED	NUMBER OF REARRANGEMENTS	% SPERMS SHOWING REARRANGEMENTS	NUMBER OF BREAKS	BREAKS PER 100 SPERMS
♂♂ ca. 5000 r	263	125	47.5±3.1	335	127.4
♂♂ ca. 5000 r+24 hrs. in ♀♀	315	158	50.2±2.8	427	135.5
		Diff./S.E.	2.7/4.2=0.64		
♂♂ 4000 r	203	63	31.0±3.2	178	87.7*
♂♂ 4000 r+72 or 144 hrs. in ♂♂	483	133	27.5±2.0	386	79.9
		Diff./S.E.	3.5/3.8=0.92		

* This includes the 34-break rearrangement described by KAUFMANN (1943).

rearrangements, was detected between the progeny obtained from eggs fertilized by spermatozoa that had been exposed to X-rays and near infrared rays, and those fertilized by spermatozoa that had been treated with only the X-rays. These results parallel those obtained in the earlier experiments, in which spermatozoa that had been treated by exposure of males to X-rays, or

to X-rays plus near infrared rays, were transferred in copulation within the first nine or ten days following termination of the X-ray treatment. Summarized data for these experiments are presented in the lower half of table 1; a detailed analysis is to be found in an earlier publication (KAUFMANN et al. 1946).

DISCUSSION

Qualitative differences among breaks induced by irradiation of spermatozoa: The data here presented, together with those obtained previously, indicate that the frequency of chromosomal rearrangements induced by X-ray treatment of the mature spermatozoon of Drosophila can be modified by near infrared radiation when it is used before the X-rays, but not when the sequence is reversed. (A similar difference between the effects of pretreatment and posttreatment was detected in experiments in which breakage was induced by the nitrogen mustard, methyl-*bis*(betachloroethyl)amine hydrochloride; KAUFMANN and GAY 1948.) This difference seems to warrant further consideration of the sequence of events that transpires between induction of breaks and utilization of the breakage ends in structural alteration.

Previous studies also showed that near infrared radiation in itself is ineffective in inducing gene mutations or chromosome breaks, and that its use in combination with X-rays does not produce any significant increase in the overall frequency of induced recessive and dominant lethals (KAUFMANN et al. 1946; KAUFMANN and GAY 1947). Cytogenetic analysis has shown that in some cases the recessive lethals are associated with rearrangements, and that in other cases they are independent of detectable aberrations. Although the frequency of rearrangements is modified by pretreatment with near infrared radiation, no parallel increase was detected among the associated recessive lethals; and this finding indicates that lethals are not dependent for their expression on the formation of rearrangements. Dominant lethals are nonviable alterations, most of which presumably result from single breaks as a consequence of end-to-end union of sister chromatids (MULLER 1940, 1941; PONTECORVO 1941, 1942; DEMEREC and FANO 1944; CATCHESIDE and LEA 1945a, 1945b). A small proportion of the dominant lethals may represent unbalanced, multiple-break rearrangements, and it has been suggested that the frequency of this class, like that of the homologous class of viable rearrangements, may be increased by pretreatment with near infrared radiation (KAUFMANN et al. 1946).

Thus the action of near infrared radiation in "sensitizing" the chromosomes of Drosophila appears to be restricted to the control of processes involved in the formation of chromosomal rearrangements. That the higher frequency of these rearrangements resulting from pretreatment is not referable to an overall or general increase in number of all types of induced breaks is indicated by the fact that no increase was observed in frequency of the single-break type of dominant lethal. This finding, considered together with the observation that near infrared radiation in itself is not a mutagenic agent, has led to formulation of the interpretation that the sensitizing action of the portion of the spectrum

centering around wave length 10,000 Å is attributable to facilitation of recombination among the breaks provided by the ionizing radiations (KAUFMANN et al. 1946; KAUFMANN and GAY 1947). It is also apparent from these observations that breaks fall into qualitatively different classes, since the subsequent behavior of some breakage ends was modified by conditions created as a consequence of pretreatment with near infrared radiation, whereas that of others was not. The question accordingly arises whether such qualitative differences in Drosophila may be ascribed to variations in the time at which the different types of aberration are produced. Although the experiments utilizing near infrared radiation do not in themselves permit a final answer to this question, they provide clues that can be evaluated in terms of the total evidence now available.

Origin of the single-break dominant-lethal aberrations: Evidence previously available indicated that breakage ends induced by X-ray treatment of chromosomes of mature spermatozoa do not participate in the formation of viable types of rearrangements until after the sperm has penetrated the egg in fertilization. This evidence was provided by MULLER (1940), who observed by genetic analysis approximately the same frequency of translocations between the second and third chromosomes whether equivalent doses of X-rays were administered continuously or in a series of widely spaced fractions; and the same result was reached by KAUFMANN (1941a), who found by salivary-gland chromosome analysis that continuous and fractionated treatments yielded comparable frequencies of breaks and proportions of different types of rearrangements. This evidence led to the conclusion that exposure to ionizing radiations produces in the chromosomes of the spermatozoon a series of breaks or potential breaks whose ability to participate in structural rearrangement depends on the availability of other similar breaks. It was assumed, moreover, that breakage ends that fail to establish contacts with other similar ends—when chromosome movement is initiated after the spermatozoon has entered the egg—may subsequently undergo restitution, to establish the original sequence of parts. By analogy the further assumption was made that breaks not recombined or restituted may remain "open" until longitudinal division of the chromosome occurs in the male pronucleus, whereupon an opportunity is provided for end-to-end union of sister chromatids to establish a dicentric chromosome with dominant lethal potentialities (MULLER 1940). The latter assumption, however, is not an essential concomitant of the experimental findings, which concern only that fraction of the total number of breaks that is involved in the production of detectable rearrangements. The additional experimental evidence now available shows that this class of breaks is subject to modification by pretreatment with near infrared radiation, whereas the group utilized in the formation of the bulk of dominant lethals is not. As an explanation of the difference, the possibility may be considered that eventuation of the single-break dominant-lethal aberrations occurs before and not after the sperm has entered the egg in fertilization.

If production of a nonviable arrangement from a single break merely rep-

resented an alternative pattern of reunion to that provided by restitution, it might be expected that the frequency of dominant lethals would be reduced as a consequence of pretreatment, because some of the breaks would be converted to the type capable of recombining, and fewer would be available for either restitution or production of arrangements with dominant lethal potentialities. No such reduction was obtained; but it must be kept in mind that the argument involves two assumptions: first, that pretreatment does not increase the store of breaks primarily induced by the X-rays, and second, that the proportion of breaks normally available for restitution is not exceedingly large as compared with the proportion recombining (calculations by CATCHESIDE and LEA 1945b, and LEA and CATCHESIDE 1945, indicate that it is not). Another line of evidence is provided by combination treatments in which ultraviolet rays were used after X-rays. It was found that posttreatment with radiation of wave length 2537 Å effected a marked decrease in the frequency of chromosome rearrangements, but no commensurate modification in the frequency of dominant lethals (KAUFMANN and HOLLAENDER 1942, 1946). Such a result might be expected if breaks utilized in the production of dominant lethals were no longer subject to modification at the time the ultraviolet treatment was administered. The conditions requisite for formation of aberrations during this period would be realized if the chromosome of the mature spermatozoon contained at least two chromonemata, and if creation of a thoroughgoing lesion was followed promptly by the union of sister chromatids end-to-end to produce dicentric bodies and acentric fragments (following the pattern described by MCCLINTOCK 1938a, 1938b for maize).

Parallel lines of evidence also leading to the conclusion that different types of rearrangements may be realized at different stages in the breakage-recombination cycle have been furnished by studies on Tradescantia. Microspores of these plants irradiated during early prophase yield a type of break that traverses simultaneously sister chromatids lying close together. If end-to-end union of strands occurs later at the sites of these isochromatid breaks, there results a type of aberration (a dicentric body with an associated acentric fragment) that is homologous with the type considered to be responsible for the major portion of the dominant lethals in Drosophila. Treatment of pollen-tube chromosomes of Tradescantia with ultraviolet rays (λ2537 Å) after X-rays inhibits production of chromatid breaks and translocations, but not of isochromatid breaks (SWANSON 1944). Similarly, posttreatment with near infrared radiation increases the frequency of chromatid breaks and exchanges, but not of isochromatid breaks (SWANSON and HOLLAENDER 1946). SWANSON has concluded from these observations that aberrations resulting from isochromatid breaks are realized immediately on X-radiation, and hence are not subject to modification by posttreatment. Results obtained from pretreatment experiments do not correspond so closely to those obtained with Drosophila, since ultraviolet radiation effects some inhibition, and near infrared radiation some augmentation of the frequency of isochromatid-break aberrations as compared with X-ray controls; but such differences between Tradescantia and

Drosophila may be referable to differences in organization of materials in the prophase chromosomes of the treated microspores and in the condensed chromosomes of the mature spermatozoa.

The interpretation that dominant-lethal aberrations of the single-break type are produced immediately after induction of thoroughgoing breaks by the ionizing radiation implies that each chromosome of the mature spermatozoon contains at least two independent chromonemata. This type of structural organization had previously been suggested by the occurrence of gene mosaics (PATTERSON 1933; MOORE 1934), and chromosome duplications (KAUFMANN 1941b). Breaks utilized in the production of the duplications sometimes occur at identical loci in the two chromatids of the irradiated chromosome, sometimes independently in each strand. These chromatid breaks in themselves do not provide unequivocal evidence that the chromosome is longitudinally double at the time of irradiation. Since the number of strands in the mature spermatozoon cannot be determined cytologically, the alternative possibility must be considered that the chromosome is single at the time of treatment, and that differences between the locations of breaks in sister chromatids are referable to differential patterns of recombination and restitution in the two strands after their separation. There is, however, abundant cytological evidence that chromosomes of some spermatozoa are multiple stranded (review in KAUFMANN 1948), and this factor together with the evidence provided by the combination-treatment experiments, and the experimental verification of the occurrence of isochromatic breaks in Tradescantia, substantiates the interpretation that isochromatid breaks may also occur in Drosophila.

Some of the duplications have shown that broken ends may establish independent patterns of reunion distal and proximal to a break (KAUFMANN 1941b). Comparable differences in the behavior of ends of an isochromatid break have been observed in Tradescantia (CATCHESIDE, LEA, and THODAY 1946a; LEA 1947; KOTVAL and GRAY 1947). Quantitative analyses have indicated, however, that such behavior is exceptional, and that in a high percentage of cases the ends proximal and distal to the break behave similarly (CATCHESIDE et al. 1946a). The low frequency of duplications of the reversed-repeat type encountered in analyses of salivary-gland chromosomes of F_1 larval progeny of treated males of Drosophila also indicates that the same pattern of reunion usually occurs proximal and distal to the break.

Union of chromatids end-to-end following the production of a thoroughgoing break in the chromosome of the spermatozoon would also explain the extreme rarity of terminal deficiencies among the quota of rearrangements that are available for salivary-gland chromosome analysis. Aberrations of this type are not necessarily lethal in Drosophila (DEMEREC and HOOVER 1936; SUTTON 1940; BISHOP 1941), and their failure to be detected cytologically is probably attributable to some pattern of behavior of the breakage ends. CATCHESIDE and LEA (1945b) irradiated ring-X chromosomes but did not detect among 749 tested the V-shaped chromosome that would arise from an intercalary break followed by healing of the broken ends. Failure of such chromosomes to appear was attributed to the low frequency with which healing of both broken ends

occurred simultaneously; but the interpretation here presented emphasizes the possibility that "healing" does not occur because the two chromatids of each chromosome unite end-to-end immediately following breakage to give rise to a dicentric double ring—an inviable, dominant-lethal type of aberration. This interpretation also obviates the necessity of postulating the existence in the chromosomes of Drosophila of a special particle, or telomere, whose presence is essential to the viability of the chromosomes (MULLER 1940).

Restitution and recombination of breakage ends: The pattern of analysis presented in the foregoing paragraphs suggests that thoroughgoing breaks provide a mechanism for the production in the mature spermatozoon of dominant-lethal aberrations, whereas other lesions of lesser magnitude persist until the sperm has entered the egg—when movement of chromosomes provides the opportunities for establishment of new associations. On this basis, breaks might be classified as "complete" or "potential." The analysis must now be extended to determine whether the total experimental data permit such simple classification. More specifically the question is whether all the breaks not utilized in the formation of dominant lethals persist until the time of fertilization. Combination-treatment experiments again serve to provide illuminating information.

It was indicated previously that ultraviolet radiation (λ2537 Å) when used after X-rays will lower the frequency of chromosomal rearrangements. This means that fewer breaks recombine after the sperm has entered the egg. If the energy supplied by the ultraviolet radiation were effective in transforming potential into thoroughgoing breaks such conversion should be reflected in a rise in the frequency of dominant lethals. Since no such rise was detected, the conclusion was reached that the effect of posttreatment with λ2537 Å is attributable to increased restitution rather than to an increase in frequency of the single-break type of derangement (KAUFMANN and HOLLAENDER 1946). A similar conclusion was reached by SWANSON (1944) from parallel studies on Tradescantia. These experiments agree in indicating that modification of the potential breaks induced by ionizing radiations may be effected by supplementary treatment in the interval between induction of breaks and formation of rearrangements.

Treatment with near infrared radiation during this period was not effective, however, in modifying the frequency of rearrangements in Drosophila. The amount of energy provided by absorption of a radiation quantum of λ10,000 Å is considerably less than with ultraviolet radiation, and this factor may account for the difference in efficiency of the two regions of the spectrum; but it must be kept in mind that near infrared was effective, when used before the X-rays, in altering the frequency of rearrangements. If all the breaks induced by X-rays maintained until the time of fertilization the capacity to participate in restitution or recombination, some modifying action of posttreatment with near infrared radiation might be anticipated. This anticipation is realized in Tradescantia, as the experiments of SWANSON referred to previously have shown. Since in Tradescantia breakage and recombination proceed concurrently, it is possible that movement of chromosomes, which is lacking in Dro-

sophila, may be the essential factor facilitating recombination in one species and not in the other. It is also possible that the potential breaks available at this period are not modifiable by the limited amount of energy provided by near infrared radiation because the more labile breaks have previously been eliminated by restitution. Some experimental data are available that bear on this problem.

In the first place it has been reported by KANELLIS (1946) that if irradiation of the mature spermatozoon is carried on at a very low temperature (ca. 2°C) a higher frequency of rearrangements is obtained than with irradiation at a high temperature. This result is in harmony with earlier ones of PAPALASHWILI (1935) and MICKEY (1939), although other experiments in which irradiation was undertaken at different temperatures within the range between 5° and 37°C showed no significant difference in frequency of rearrangements (MULLER 1940; KAUFMANN unpublished data). KANELLIS suggests that less restitution takes place at low than at high temperature, so that more breaks are available for recombination, and this in turn implies that restitution may be occurring even during the period of X-ray treatment.

A comparable increase in frequency of chromatid breaks and chromosome aberrations has been obtained in Tradescantia by irradiating microspores at low temperature. This result has likewise been attributed to delay of restitution (SAX and ENZMANN 1939). More recent evidence indicates that the temperature factor operates primarily or exclusively during irradiation, even when it is restricted to a period of 80 seconds (FABERGÉ 1948; cf. also SAX 1947; CATCHESIDE, LEA, and THODAY 1946b). If the increase in frequency of rearrangements is attributable to inhibition of restitution, such restitution must normally occur immediately after the breaks are induced, because the temperature-treatment period is relatively short as compared with the time the breaks remain "open" (average ca. 4 minutes according to calculations of LEA and CATCHESIDE 1942, although recent data by SWANSON, 1947 indicate that some restitution may be delayed as long as 4 hours after irradiation).

These studies on Drosophila and Tradescantia thus suggest that restitution may take place in some of the potential breaks before the time of recombination. Inhibition of restitution during this period should increase the store of potential breaks available for subsequent recombination; and perhaps the "sensitizing" action of pretreatment of chromosomes with near infrared radiation is attributable to changes in the quality of potential breaks that prevent such post-irradiation restitution (cf. also the recent report of FABERGÉ (1948) that change of temperature in either direction immediately preceding irradiation increases the number of aberrations in Tradescantia).

A considerable portion of the potential breaks must nevertheless persist until movement of chromosomes provides opportunities for recombination. The opportunities may be increased by supplementary treatment; for example, it has been found in Drosophila that treatment with near infrared radiation at the time of syngamy is effective in increasing the frequency of rearrangements (KAUFMANN 1946). Potential breaks that fail to establish new contacts during

the period of recombination presumably undergo restitution soon thereafter, although some experimental evidence suggests that recombination may be delayed under certain conditions until after the first cleavage mitosis (HELFER 1940).

Recapitulation: An analysis of frequencies and types of rearrangements provided by experiments in which supplementary agents were used in combination with ionizing radiations in treatment of the mature spermatozoon of Drosophila indicates that breaks fall into qualitatively different groups that may be classified tentatively as "complete" and "potential." These differences are reflected in the subsequent behavior of the breakage ends.

It is suggested that the complete, or thoroughgoing, breaks provide the opportunity for formation within the chromosomes of the mature spermatozoon of aberrations involving end-to-end union of sister chromatids, and that these eventuate as dominant lethals. The further suggestion is made that some of the potential breaks are restituted during the period of irradiation or shortly thereafter, whereas others persist until opportunities are offered for recombination after the spermatozoon has entered the egg in fertilization. Although based on an appraisal of available data, these suggestions concerning the time of realization of different types of aberrations must be regarded as tentative, pending the accumulation of additional experimental evidence that will help resolve some of the existing perplexities. Why, for example, if the chromosome of the mature spermatozoon is longitudinally double, do both chromatids together usually behave as a unit in establishing new connections at the time of restitution? In the absence of the definitive evidence necessary to answer such questions, a reevaluation of the data now available has served to focus the problem of structural rearrangement on the analysis of the behavior of individual breakage ends. As MCCLINTOCK (1941) concluded from studies on maize, the factors responsible for fusion or restitution are probably related not only to the method of origin of the breaks but to the physiological conditions surrounding the broken ends.

Of the various types of supplementary treatment utilized, the near infrared portion of the spectrum has proved most serviceable in analysis of the cycle of breakage and recombination in Drosophila. Studies on Drosophila indicate that near infrared radiation can modify the processes that lead to structural rearrangement of chromosomes, but not those responsible for gene mutation. Accordingly, this type of radiation may serve as a useful diagnostic tool, if applied to organisms in which cytological analysis is not feasible, in distinguishing between genetic changes that are attributable to chromosomal alterations and those that are not. It was reported following the earlier studies on Drosophila that the "sensitizing" action of near infrared radiation is not attributable primarily to an over-all or general temperature effect, but to a more specific influence of the radiation on certain materials of the chromosome; and this point of view has been substantiated by SWANSON'S experiments on Tradescantia. The potentialities of this portion of the spectrum for further experimental work lie, therefore, in the prospect of securing intense mono-

chromatic radiation of known physical properties. Experiments directed to this end are now in progress.

SUMMARY

Exposure of mature spermatozoa of *Drosophila melanogaster* to near infrared radiation after treatment with X-rays did not effect an increase in the frequency of rearrangements similar to that obtained when the near infrared preceded the X-rays. A comparison of these results and those obtained in previous experiments suggests that breaks induced by ionizing radiations may be qualitatively different—with respect to their potentialities for subsequent behavior—from the time of their inception. It is suggested that some breaks are thoroughgoing and therefore provide immediate opportunities for union of sister chromatids end-to-end to produce dicentric bodies and acentric fragments which result in dominant lethals. It seems probable that the close proximity of chromatids in the compact sperm head facilitates sister union and inhibits thereby the formation of a class of dominant lethals characterized by "healing" of breakage ends and the production of terminal deficiencies. Less damage to the chromosome presumably occurs at other breaks, at which restitution may be effected either during the time of X-ray treatment or shortly thereafter; or the breaks may remain "open" until opportunities for recombination are provided after the spermatozoon has penetrated the egg in fertilization.

The analysis also indicates that the sequence of events which transpires between irradiation and the production of aberrations may not differ so greatly in Drosophila and Tradescantia as has previously been assumed. If some restitution occurs prior to the time of recombination—and not merely as a consequence of failure of recombination, as has generally been assumed—the essential difference in the breakage-recombination cycle in Tradescantia and Drosophila lies in the fact that movement of chromosomes to facilitate recombination may occur in the cells of Tradescantia during or shortly after exposure to the ionizing radiation, whereas such movement is not possible in Drosophila until the attenuated sperm head is transformed into the spherical male pronucleus at the time of fertilization.

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THE STRUCTURE AND MEIOTIC BEHAVIOR OF THE DIFFERENTIATED CHROMOSOMES OF TOMATO

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FOR a number of years the elongate chromosomes of salivary gland nuclei of Diptera and the pachytene chromosomes of plants have proved of use in researches in cytogenetics. The pachytene and later meiotic chromosomes of the tomato offer interesting material for cytological and perhaps cytogenetic studies because of their morphology. The chromosomes of the tomato are strikingly differentiated into regions differing in diameter and staining capacity. In addition, the differential regions seem to show differential behavior during meiosis with respect to pairing, location of chiasmata, and contraction.

The arms of the tomato chromosomes are divided into two sorts of regions. One sort is adjacent to the centromere, is relatively broader, and stains very deeply with iron aceto-carmine. Because of its staining capacity, this sort of region will be called a chromatic zone. The other sort forms the distal portion of the chromosome arms, is relatively narrower, and stains very lightly. This sort of region will be called an achromatic zone. The chromatic zones have a very distinct pattern of specific chromomeres while the achromatic regions possess very delicate chromomeres which are difficult to observe. The transitions from the chromatic to the achromatic zones are very abrupt. The achromatic regions all terminate in small, but very distinct knobs, or telochromomeres which thus form the ends of the chromosomes.

The chromosomes of the tomato seem to correspond most closely in structure to the meiotic chromosomes of Agapanthus (BELLING 1928; DARLINGTON 1933; GEITLER 1933), Oenothera (JAPHA 1939; MARQUARDT 1937), Pellia (JACHIMSKY 1935), and Sphaerocarpus (LORBEER 1934). Each of the features mentioned above for the tomato has been reported for one or more of these plants. It is possible to see all of these characteristics clearly in the tomato.

Because of its importance as an economic plant, the tomato has been the subject of numerous cytological investigations. Although achromatic zones have been recognized at pachytene (LESLEY and LESLEY 1935) and achromatic threads at diakinesis pictured by many workers, all previous investigators have concerned themselves largely with the chromatic material. Technical difficulties have apparently prevented previous workers from obtaining a clear picture of chromosome structure in the tomato. Some of these difficulties have recently been overcome by the development of a mordanting modification of the aceto-carmine smear technique by DR. MARTA S. WALTERS. Even with this method which gives an unusually intense stain to the chromatic zones, the

achromatic zones are occasionally so poorly stained that they are difficult to observe.

In the following report the structure of the chromosomes at pachytene will be described first. After this, the behavior during the meiotic sequence, with respect to pairing, formation of chiasmata, and contraction, will be described.

MATERIALS AND METHODS

All plants used in this study were from a highly inbred line of the English greenhouse forcing variety, Sutton's Best. Some of the plants originated from X-rayed pollen, the 700-series, and the others were untreated, the 800-series. The irradiation was performed to produce a translocation which was necessary for part of the study of chromosome contraction, and this translocation was studied in the single F_1 plant, 725-1. Studies of the nucleolus-organizing chromosome were made with three plants, 808-18, 725-1, and 724-1; the 700-series plants were used inasmuch as good preparations were available, and the paired homologues at pachytene showed no detectable aberrations. Measurements of the nucleolus-organizing chromosome were made of at least several chromosomes at pachytene in all plants from which later stages were taken. Studies of chromosome pairing at zygotene and of chiasmata frequencies at diakinesis were made only on untreated material in order not to confuse the normal behavior with that which might be caused by aberrations.

Petals and sepals were removed from buds before they were fixed for twenty-four hours in three parts absolute alcohol to one part glacial acetic acid. The writer wishes to express his appreciation to DR. MARTA S. WALTERS for information on the subsequent technique. This method was adapted to the tomato by her, and has given excellent results in this and other laboratories. After fixation the buds were soaked in two or three changes of tap water for thirty minutes to an hour. They were then mordanted for thirty minutes to an hour in four percent iron alum after which they were again soaked in several changes of tap water for at least an hour. After this mordanting the buds would not keep well and were used at once. Contents of an anther were squeezed out into a small drop of iron aceto-carmine, the debris removed, and the pollen-mother cells broken up by stirring with a needle. A cover-slip was placed on top of the drop, and the slide was heated over an alcohol burner until it was hot to the touch but not boiling. The slide was then placed between several layers of paper toweling and pressed vigorously, with care not to move the cover-slip. Slides can be sealed with a convenient preparation or made permanent in euparal (DARLINGTON and LACOUR 1942). All photomicrographs in this report were made from permanent preparations.

Photomicrographs were made with a Bausch and Lomb K camera which gave an enlargement, with a 90× objective and 15× ocular, of 1200×. Enlargements to 2000× or greater were made with an Omega DII enlarger. All negatives were Eastman Contrast Process Ortho, developed in D-11. Most of the papers used in printing were F4 and F5.

PACHYTENE STRUCTURE

The structure of the tomato chromosome is most clearly evident at the pachytene stage of meiosis. The chromosomes typically show the following structural characteristics: (1) a centromere, (2) chromatic regions on each side of the centromere and of varying extent for each arm, (3) achromatic distal regions, also of varying length, and (4) small terminal knobs, or telochromomeres, which appear to terminate all chromosome arms. These characteristics will be considered in detail.

The chromatic and achromatic zones appear to possess some of the properties of hetero- and euchromatin, respectively. The behavior of the chromatic material in the resting nucleus is complex. Consequently the simple descriptive terms will be used in this report, and some of the similarities of the regions of the tomato chromosomes to hetero- and euchromatin as they have been described in other organisms will be referred to in the discussion.

The centromere resembles that of maize in being an elongate and achromatic structure (McCLINTOCK 1931). In some cells all twelve centromeres may be seen, but will show more clearly in certain chromosomes than in others (figs. 2, 6, chromosomes 6, 7, 8, 9, 10, 11, 12).

The chromatic zones appear to consist of relatively large chromomeres which stain deeply. Between these large chromomeres, which vary somewhat in size along the length of the chromosome, narrower achromatic zones are sometimes apparent. The two chromosomes most readily identifiable at pachytene are the nucleolus-organizing chromosome and a chromosome with a very short and characteristically patterned chromatic zone. Both will be used to illustrate the structure of the chromosomes. The nucleolus-organizing chromosome has a short arm which is completely chromatic and a long arm consisting of a chromatic zone and a very long terminal achromatic region. The long arm begins with a very heavy chromomere adjacent to the centromere; this heavy chromomere is followed by a less heavily chromatic zone which is followed again by large chromomeres (figs. 3, 7, 9, 22). The chromatic zone of the chromosome with the short chromatic region consists of only three chromatic elements, a very large chromatic structure which begins the short arm and two heavy chromomeres in the proximal part of the long arm (figs. 2, 6, 8, 21).

The achromatic zones of the tomato chromosomes constitute the distal portions of all the chromosome arms except the short arm of the nucleolus-organizing chromosome. They are very weakly stained threads which in many cases are difficult to differentiate from the cytoplasm in which they are usually spread out on smearing (figs. 8, 21). The boundary between the chromatic and achromatic zones is usually sharply defined. The chromomeres of the narrow transition regions between the chromatic and achromatic zones are stained deeply but are distinctly smaller than those of the chromatic zones (figs. 2, 6, upper arms of chromosomes 1 and 12). The achromatic regions possess chromomeres which are very difficult to detect because of their light stainability (figs. 42–49). No chromosome has been observed which is not achromatic for at least half its length. The chromosome with the short chromatic region obviously has

a much greater achromatic than chromatic length (figs. 2, 6, 8, 21). The same is true of the nucleolus-organizing chromosome (table 1, figs. 3, 7, 9, 22). Other chromosomes may possess obviously shorter achromatic regions (figs. 3, 7).

The chromosomes typically end with a small chromatic knob, or telochromomere. The telochromomeres are smaller than the chromomeres of the chromatic zones and must be in sharp focus to be seen. For example, the telochromomere at the end of the short arm of the chromosome with the short chromatic region is clearly visible in one photomicrograph (figs. 2, 6), but not in the other (figs. 8, 21). The demonstration that all twenty-four chromosome arms do end in telochromomeres will require the identification of all twelve chromosomes. However, over seventeen ends with telochromomeres have been counted in single nuclei and the five lowermost achromatic zones in figs. 2 and 6 may all be seen to terminate in telochromomeres. In addition, no chromosome end which has been seen clearly has been found not to possess a telochromomere. Thus, if not a universal feature of the chromosome ends of the tomato, the telochromomere is at least very typical.

The region of the nucleolus-organizing chromosome associated with the nucleolus is in the short arm. This arm is apparently the only one of the complement which consists entirely of chromatic material. In mitotic divisions (figs. 30–34) the distal end of this arm is clearly dissociated from the rest of the chromosome to form a small satellite. In meiosis, on the other hand, the distal end comes to lie adjacent to the remainder of the short arm, and no separate satellite is formed. The region connected with the nucleolus can be identified because the rest of the chromosome is frequently pushed away from the nucleolus on smearing. A shallow and short constriction may sometimes be seen at this region, and beyond this constriction the distal end of the short arm frequently appears as a lump or bump. How much of this distal end is concerned with the formation of the nucleolus is not known at present. The only point which can be determined with certainty is the region closest to the centromere to which the nucleolus is attached. This is the region marked by the shallow constriction. The distal end of the chromosome which occurs beyond the point where the nucleolus is attached to the chromosome may be seen in figs. 6 and 7, and is diagrammed as the solid region in figs. 2 (chromosome 3) and 3. The length of the distal segment is 1.9 microns at pachytene, or about 40 percent of the length of the short arm (table 1). In mitotic prophase and metaphase, the satellite formed about 50 percent and 42 percent of the lengths of the short arms, respectively. LESLEY and LESLEY (1935) and LESLEY (1938) have been concerned in particular with the satellite region of the chromosome, and have discovered varieties of tomato differing in satellite size. The satellite of Sutton's Best seems definitely to belong to the "short" rather than to any of the "long" classes.

ZYGOTENE PAIRING

In cells of late zygotene or early pachytene in which pairing is only partially completed, the chromosome regions seem to be differentially paired according to their chromaticity. Such cells are not abundant, so the stage in which the

pairing differential may be observed clearly is probably of short duration. However, there are usually several chromosome arms showing this behavior in one nucleus. Both the centromere and the distal achromatic zones pair while the chromatic zones usually remain completely or partially unpaired (figs. 1, 5).

TABLE 1

Lengths in microns of regions of the nucleolus-organizing chromosome during meiotic and mitotic stages (see also diagrams, fig. 51)

STAGES	REGION						
	SATELLITE*	TOTAL SHORT ARM	CENTROMERE	CHROMATIC OF LONG ARM	TOTAL CHROMATIC**	ACHROMATIC OF LONG ARM	TOTAL CHROMOSOME
Pachytene	1.9	4.8	1.6	4.6	11.0	26.9	37.9
S.E.***	(10)	*0.21*	*0.15*	*0.15*	*0.36*	*1.31*	*1.33*
Early Diakinesis	—	—	—	—	3.8	6.2	10.0
S.E.	—	—	—	—	*0.24*	*0.32*	*0.33*
Middle Diakinesis	—	—	—	—	2.3	4.2	6.5
S.E.	—	—	—	—	*0.10*	*0.26*	*0.30*
Late Diakinesis	—	—	—	—	1.9	2.9	4.8
S.E.	—	—	—	—	*0.06*	*0.12*	*0.13*
Interphase	—	1.2	0.5	1.2	2.9	0.0	2.9
S.E.	—	*0.08*	*0.07*	*0.08*	*0.15*	—	*0.15*
Mitotic prophase	0.9	1.7	0.0	0.9	2.6	1.6	4.2
(n)***	(4)	(2)	(4)	(2)	(4)	(2)	(2)
Mitotic metaphase	0.6	1.4	0.0	0.9	2.3	0.0	2.3
(n)	(2)	(2)	(2)	(2)	(2)	(2)	(2)

* The "satellite" measurements for pachytene are the lengths from the apparent region of attachment of the nucleolus to the distal end of the short arm.

** The lengths of the centromere are included in all total chromatic lengths because the centromere can not be distinguished at later meiotic stages.

*** The standard error is given in italics below each of the averages; n = 15 for pachytene, and n = 25 for later meiotic stages. Where standard error is not given, the number of measurements made, n, is given in parentheses.

Later stages, of course, show an apparently tight synaptic union along the entire length of the chromosome.

The observed interruptions in pairing, however, may be thought to be the result of the usual sequence of chromosome pairing in the tomato without respect to any possible differential behavior of the chromatic regions. In order to determine whether or not chromatic differentiation plays a part in chromosome pairing, the results obtained from observations on pairing were compared statistically with the results expected on the basis of several hypotheses of chromosome pairing. These hypotheses assume that pairing is independent of chromaticity.

Cells were studied in order from a slide with late zygotene material on it. The first 40 chromosome arms having unpaired segments were measured with

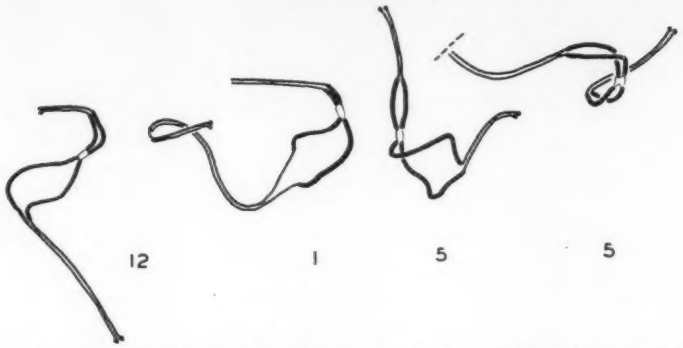

FIGURE 1.—Diagram of fig. 5. Late zygotene or early pachytene. Four bivalents, at 12, 1, and two at 5 o'clock in the photograph. Part or all of the chromatic material of each bivalent is not yet paired while achromatic regions and centromeres are paired.

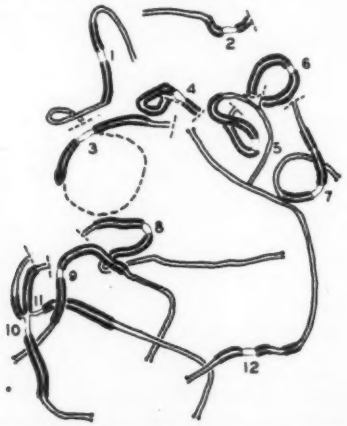

FIGURE 2.—Diagram of fig. 6. Pachytene. All twelve chromatic regions are apparent. Chromosomes are numbered for reference purposes only, and the numbers do not imply identification of all of the chromosomes. Note the chromosome with the short chromatic region, No. 12; it may be followed its entire length. No. 3 is the nucleolus-organizing chromosome; only its chromatic material is visible. Nos. 10 and 11 show centromeres lying close together.

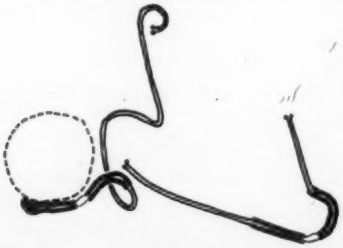

FIGURE 3.—Diagram of fig. 7. Pachytene. The nucleolus-organizing chromosome and one other may be followed their entire lengths.

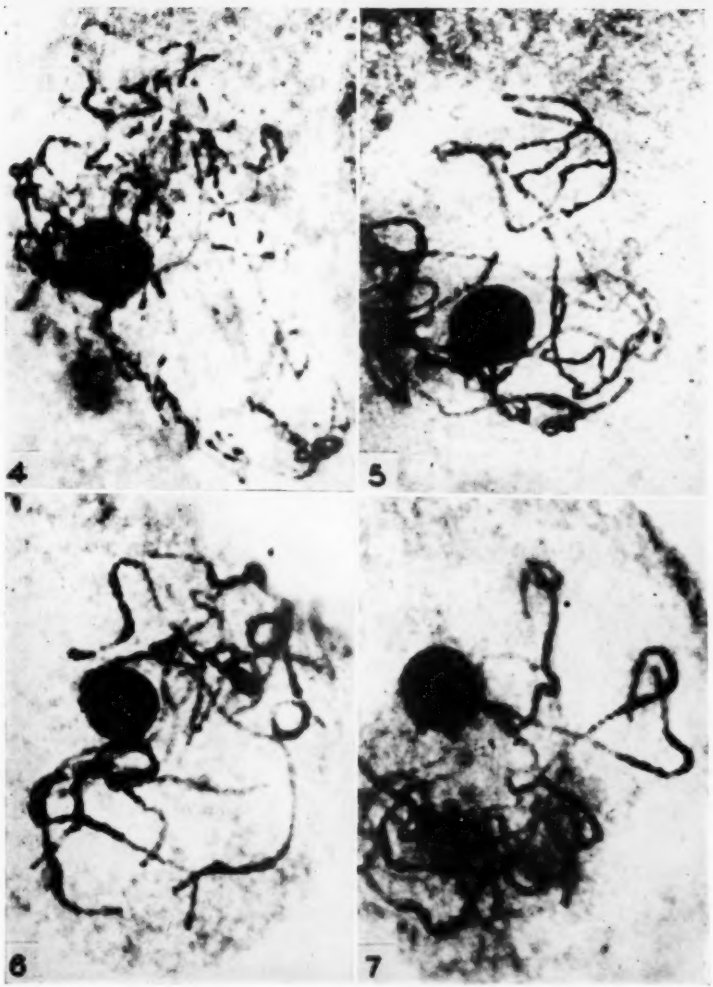

PLATE I

Zygotene to pachytene. 2000×. Fig. 4. Zygotene. Fig. 5. Unpaired chromatic zones at early pachytene. See diagrams, fig. 1. Fig. 6. The chromosome with the short chromatic region at pachytene, chromosome No. 12, see diagram fig. 2. All twelve chromatic regions are visible. Fig. 7. Nucleolus-organizing chromosome and another bivalent at pachytene. See diagram, fig. 3.

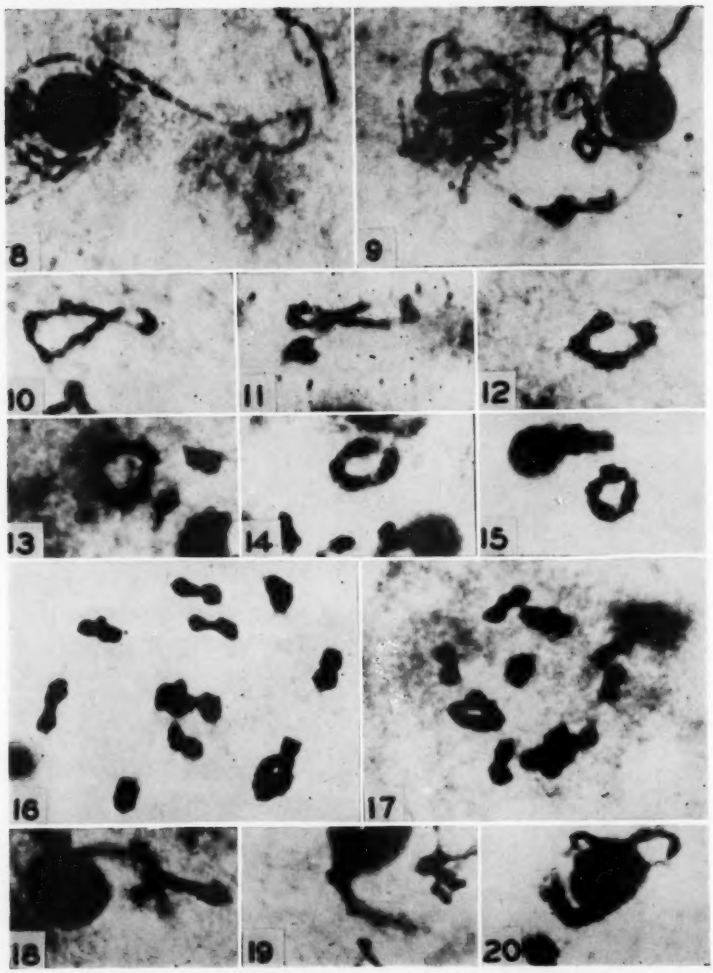

PLATE II

Pachytene to prometaphase. 2000×. Fig. 8. The chromosome with the short chromatic region at pachytene. See diagram, fig. 21. Fig. 9. Nucleolus-organizing chromosome at pachytene. See diagram, fig. 22. Figs. 10–11. Translocation ring-of-four chromosomes at early diakinesis. Figs. 12–14. Translocation at middle diakinesis. Fig. 15. Translocation at late diakinesis. Figs. 16–17. Translocation at prometaphase with rest of complement, at five o'clock in fig. 16 and at eight o'clock in fig. 17. Figs. 18–20. Nucleolus-organizing chromosome at early diakinesis.

an ocular micrometer. For each of these arms, the lengths of the chromatic and achromatic zones, and the length and position of the unpaired segment were recorded. The results of these measurements are illustrated graphically in fig. 23. In the construction of this graph, each chromosome arm was given a standard length divided equally into chromatic and achromatic zones regardless of the actual lengths or proportions of these regions. The horizontal lines indicate

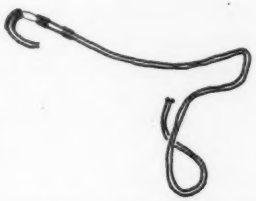

FIGURE 21.—Diagram of fig. 8. Pachytene. The chromosome with the short chromatic region. Note that in the loop, the achromatic material is difficult to differentiate from the cytoplasm.

FIGURE 22.—Diagram of fig. 9. Pachytene. The complete nucleolus-organizing chromosome.

the relative portions of the chromatic and achromatic regions which formed the unpaired segments.

Thirty-seven of the forty arms showed unpaired segments at least in part in the chromatic zones. In 30 of the 40 arms, the distal end of the unpaired segment was at or near the transition region of the chromatic and achromatic zones. In these 30 arms, pairing ends near the transition region within ten percent of the chromatic zone or ten percent of the achromatic zone. In the further discussion these regions will be referred to as the ten percent transition regions; their limits are indicated by the vertical dotted lines in fig. 23, and the 30 arms make up the group enclosed by the bracket.

It is now necessary to determine whether the occurrence of 30 distal ends of unpaired segments at or near the transition zones is or is not in reasonable agreement with the postulate that the tendency of a region to pair is independent of its chromaticity. Expected frequencies of this occurrence were therefore computed on the basis of the different assumptions described below. All the assumptions lead to expected frequencies less than those observed.

According to the first assumption, pairing takes place at random along the length of the chromosome. In this event, the probability that the distal end of an unpaired segment in any chromosome arm will be observed to be in a given region is directly proportional to the length of this region. In addition, the

assumption is made that the positions of pairing of different chromosome arms observed in the same cell are uncorrelated. According to this assumption the distal end of only one out of ten unpaired segments would be observed within the ten percent transition regions. However, unpaired segments entirely within the achromatic zones are more difficult to detect than those at least partly within the chromatic zones. Even if lack of pairing was never detected in distal regions of the achromatic zones, however, not more than one out of five of the non-paired segments observed should have its distal end at or near the transition region.

According to the second assumption, chromosome pairing begins simultaneously at the ends of the chromosome arms and moves toward the centromere regardless of the pairing behavior of the centromere itself. As pairing progresses the unpaired segments would decrease in length until, at different times for different chromosome arms, the distal ends of these segments would come to lie in the ten percent transition regions. At a particular stage at which the cells were fixed, there would be a maximum number of these ends lying within the ten percent transition regions. The expected frequencies, to be discussed below, were computed on the extreme assumption that all the cells were fixed at exactly this most favorable stage.

According to the second assumption, part *a*, pairing may proceed on a relative basis. When one chromosome arm is paired for half its length, other chromosome arms are also paired for about half their lengths regardless of dissimilarities in actual length. From the measurements of the lengths of the same 40 chromosome arms on which pairing observations were made, it was possible to determine that the largest number of transition regions, 17, occurred within the ten percent interval of 57 to 67 percent of the length of the arm from the end to the centromere. Therefore the maximum number of distal ends of unpaired segments occurring at or near the transition region, under the extreme assumption mentioned, would be expected to be 17 out of 40. If some or all of the cells were fixed at a somewhat different stage, the expectation would be lower.

According to the second assumption, part *b*, pairing starts simultaneously in all arms and proceeds at equal rates on the basis of actual length. When one chromosome arm is paired for two microns from the end, other chromosome arms are paired for approximately two microns regardless of whether two microns is a small or large part of the chromosome arm. Ten percent of the average length of all the arms, 1.4 microns, was chosen as the test interval. It was then possible to determine that the largest number of transition regions, 14, occurred within the ten percent interval of 7.2 to 8.6 microns from the end toward the centromere. The number of distal ends observed to be lying within 0.7 micron to either side of the transition region was 27.

On the basis of the first assumption, the maximum expectation of eight distal ends within and 32 without the ten percent transition region can be compared with the observations of 30 and ten, respectively. A chi square test indicates that the deviations are far greater than can reasonably be attributed to chance (table 2).

On the basis of assumptions 2a and 2b the expected values are 17 and 23, and 14 and 26, respectively. The corresponding observations are, in the same order, 30 and 10, and 27 and 13. For various reasons, a valid chi square test can not be made to compare these observed with expected values. To do so would require that each observation was independent. A chi square test would be permissible (1) were each chromosome arm observed in a different cell, (2) if the expected values were derived from the entire chromosome set, and (3) if each chromosome arm had an equal probability of being selected from a given cell. In this case, however, deviations from these requirements would tend either to decrease or not affect chi square. The observation of more than one chromosome arm in a given cell would tend to decrease chi square since if a

TABLE 2

Tests of hypotheses of chromosome pairing which assume independence of chromaticity. For further explanation, see text

DISTAL END OF UNPAIRED SEGMENT	HYPOTHESIS 1		HYPOTHESIS 2a		HYPOTHESIS 2b	
	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.
In ten percent transition zone	10	32	10	23	13	26
Elsewhere in chromosome arm	30	8	30	17	27	14
Chi square (Yates' corr. used)	72.22		15.98		17.17	
Chi	8.50		3.99		4.14	
P<	1×10^{-9}		0.000, 1		0.000, 1	

first observation showed a distal end within a ten percent transition region, the second would have a decreased probability of showing the same thing, and so forth. Secondly, since the expected frequencies are based on the actual chromosomes examined rather than on a complete set of chromosomes, the actual chi square is likely to be small. Finally, under the present circumstances, an accumulation of the same or similar arms in successive cells would increase the expected frequencies at the same rate as the observed. Thus the chi squares computed to test hypotheses 2a and 2b are to be regarded as minimum values, and the probabilities as maximum values rather than valid estimates of these values (table 2). The very low values of the two probabilities, both less than 0.0001, indicate that assumptions 2a and 2b, together with the extreme assumption that all cells were fixed at the same most favorable stage, are not tenable.

Because of the marked deviations of the observed values from those expected on the basis of all the hypotheses given above, it seems unlikely that an hypothesis which does not include the differential behavior of the chromatic and achromatic regions will satisfactorily explain chromosome pairing in the tomato.

In addition to pairing before the adjacent chromatic zones, the centromeres show one other type of differential behavior. Centromere regions of non-homologous chromosomes were frequently found to be adhered or lying on top of each other at pachytene, but not at later stages. Such behavior was noted in both X-rayed and untreated material (figs. 2, 6, centromeres 10, 11). In addition, this behavior made necessary a postponement of a study of the translocation at pachytene because the translocation seemed to occur near the centromere regions of both chromosomes, and to bring the non-homologous centromeres close enough together to induce a very frequent adherence. There was insufficient material available to differentiate the translocation configurations from those caused by adhering centromeres. In Agapanthus, DARLINGTON (1933) observed similar non-homologous centromere fusions which he believed resulted from an interlocking during pairing. Such interlocking seems not to be true in tomato because no interlocking configurations were seen to occur at later stages, and in many cases the centromeres were merely lying close together.

CHIASMATA AT DIAKINESIS AND METAPHASE; THE CHROMOSOMES AT METAPHASE AND ANAPHASE

In the tomato, chiasmata may be observed readily at late diakinesis. In order to determine their approximate number and position, all of the chiasmata in ten cells at late diakinesis were recorded. This count gave a total of 162 chiasmata of which 49 were interstitial and 113 terminal. This material therefore had an average of 1.35 chiasmata per bivalent, and a terminalization coefficient of about 70 percent. The nucleolus-organizing chromosomes of these cells, when considered alone, had nine interstitial and four terminal chiasmata, or an average of 1.3 chiasmata per bivalent and a terminalization coefficient of only 30 percent.

A single cell in which all of the chiasmata may be seen is pictured in fig. 41. In this cell there was a total of 18 chiasmata, 13 of which were terminal (T) and 5 interstitial (I). In this cell, as in the counts given in the preceding paragraph, the number of chiasmata classified as interstitial was a minimum. For example, the bivalent at eleven o'clock in fig. 41 may have had an interstitial instead of a terminal chiasma at its left end.

In the cells examined, all of the chiasmata observed were in the achromatic regions. In order to test the hypothesis that chiasmata are more abundant in the achromatic than in the chromatic regions, another series of observations was made on the short arm of the nucleolus-organizing chromosome which consists entirely of chromatic material. This region of the chromosome is difficult to work with because of the attachment to the nucleolus and because of the occasional presence during most of diakinesis of fine chromatic strands which connect the two short arms. These fine chromatic strands usually appear to be subterminal, and therefore they cannot be confused with chiasmata when they are in this position. At late diakinesis, the association of the chromosome with the nucleolus has considerably weakened, and most of the chro-

matic strands have disappeared. Those strands which do remain are difficult to distinguish from chiasmata because the contraction of the chromosome makes them appear to be terminal.

The length of the short arm of the nucleolus-organizing chromosome at pachytene is 4.8 microns while that of the long arm is 31.5 microns. If the number of chiasmata formed is proportional to chromosome length, then the short arm of the nucleolus-organizing chromosome would be expected to have $(4.8/31.5)\times1.3$ or an average of 0.2 chiasmata per bivalent. Even if the observed chromatic strands are counted as chiasmata, the number of chiasmata in the short arm of the nucleolus-organizing chromosome falls significantly

TABLE 3

Test of significance of difference between number of chiasmata (or chiasma-like chromatic strands) in the short arm of the nucleolus-organizing chromosome at late diakinesis and the number expected on the basis of pachytene length

	OBS.	EXP.
Chromosomes separate	54	44
Chromosomes superimposed	(3)	—
Chiasma or chromatic strand	1	11
Totals	55 (58)	55
Chi square (Yates' corr. used)	10.25	
P<	0.01	

short of this expectation (table 3). Although it may be disputed whether or not chiasmata are formed at all in the chromatic zones, it seems very unlikely that they are to be found there with the same frequency, on a length for length basis, in which they are found in the achromatic regions.

The interstitial chiasmata persist until metaphase (figs. 36–39). During the early anaphase movements of separation of the bivalents, part of the chromosomes are stretched out between the separating members. As the chromosomes move still further apart, the material which previously appeared to be considerably extended between them disappears suddenly as though it had been released from tension (figs. 38, 39). At middle anaphase, the chromosomes appear more uniform than at previous stages of meiosis, and can only occasionally be differentiated into lighter and darker staining regions (fig. 40).

CHROMOSOME CONTRACTION; MITOTIC CHROMOSOMES

In the tomato, the achromatic regions seem to constitute only a small portion of chromosome length at late prophase of either mitosis or meiosis. The achromatic regions at these stages occupy a much smaller proportion of the

total length of the chromosomes than they do at earlier meiotic stages. The relatively greater shortening of the achromatic zones may be accounted for in several ways. The contraction of the achromatic zones may be proportionately greater than that of the chromatic zones. By the term contraction is meant simply a shortening or reduction in length of a chromosome or section of a chromosome. This term is not meant to imply any mechanism, such as spiralization, which might be responsible for the reduction. On the other hand, the regions of the achromatic zones near the chromatic material may change during prophase, become deeply stainable, and thus become indistinguishable in appearance from the chromatic material. If this were true, the residual achromatic material would obviously occupy a relatively smaller proportion of the length of the late prophase chromosome than would the chromatic material and the altered achromatic material together. Measurements of a chromosome with visible marker points were used to determine which of these alternatives was occurring during prophase.

The expected results from each of these two types of change is illustrated diagrammatically in fig. 50. If the achromatic material were to become reduced in length to practically nothing while the chromatic material were only moderately reduced in length, the asymmetric chromosome pictured above would eventually change to a symmetric chromosome (arrow A). If the achromatic material were to become indistinguishable from the chromatic material, while both it and the chromatic material were contracting, the resultant chromosome should be markedly asymmetric (arrow B).

The nucleolus-organizing chromosome is composed of two arms, a short, chromatic arm, and a long arm containing a short, proximal, chromatic region and a long achromatic zone. The two chromatic regions on either side of the centromere are of about equal length, while the distal achromatic zone is much longer than the two chromatic zones added together (figs. 3, 7, 9, 22; table 1). In addition, the part of the chromosome connected with the nucleolus marks off a distal knob or bulge occupying more than one-third of the end of the short arm. The limits of this distal region could not be accurately determined in all of the chromosomes which were selected for measurement, but sufficient measurements were made to give a fairly good estimate of its size (table 1). As previously mentioned, this region of the chromosome does not form a satellite at meiosis, but forms a very distinct satellite during mitosis.

During diakinesis, the chromatic region forms one end of the nucleolus-organizing chromosome, but can no longer be marked by either the satellite or the centromere (figs. 18–20, 24–27). At interphase, however, and at mitotic prophase and metaphase, the centromere region is clearly apparent (figs. 28, 29, 32, 34). If the achromatic material has really contracted to an extremely small length, the nucleolus-organizing chromosome should have arms of approximately equal length at interphase and at mitotic metaphase. In this case the chromatic material alone would form a symmetric chromosome. If the achromatic material had become altered to resemble the chromatic material, the final product should be an asymmetric chromosome with the arm bearing the nucleolus much shorter than the other.

Measurements of nucleolus-organizing chromosomes at interphase indicate that the two arms are of approximately equal length (table 1). At mitotic metaphase, the arm bearing the satellite is somewhat longer than the opposite arm. The satellite does not form a thread of the same thickness as the rest of the chromosome, and it seems for this reason that the nucleolus-bearing arm is longer at these stages. The achromatic material can be seen during mitotic divisions for other chromosomes as well as the nucleolus-organizing chromosome (fig. 35). The changes in length of the various regions of the nucleolus-organizing chromosomes may be easily summarized in a chart (fig. 51). The change from a marked asymmetry at pachytene to a nearly symmetric chromosome at interphase and mitotic metaphase indicates that the long achro-

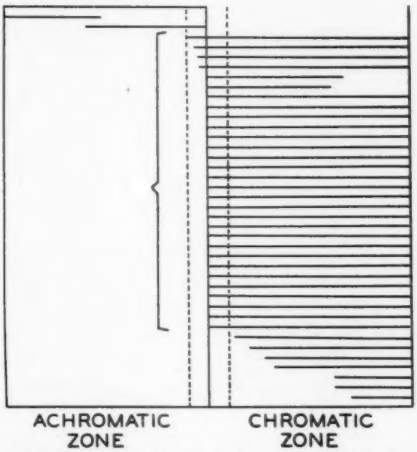

FIGURE 23.—Chart showing extent of unpaired segments in 40 chromosome arms at late zygotene. All chromosomes have been given a standard length divided into equal chromatic and achromatic zones. The horizontal lines indicate the proportionate extent and location of the unpaired segments. The vertical dotted lines indicate the ten percent of the chromatic zone and the ten percent of the achromatic zone adjacent to the transition between the two zones (continuous vertical line at center). The bracket includes the 30 arms which had distal ends of unpaired segments occurring within the ten percent limits.

matic zone has contributed practically nothing to the length of these chromosomes. Therefore, the achromatic material apparently has not acquired, either from its own production or from the chromatic zone, more than a very small amount of stainable material.

By following both the nucleolus-organizing chromosome and the translocation, the relative rate of change of length of the chromatic as compared with the achromatic zone may be determined. When lengths of chromatic regions are plotted against achromatic regions, an approximately straight line relationship is apparent, and a straight line may be easily fitted to the points by means of least squares (fig. 52). The much wider spacing of the stages for the trans-

location than for the nucleolus-organizing chromosome follows from the fact that the translocation measurements include four chromosome lengths (two bivalents) while those of the nucleolus-organizing chromosome, only one (one chromosome from each bivalent) (figs. 10–17). The chromosomes in the translocation seem to be smaller than the nucleolus-organizing chromosome.

The slopes of the two lines in fig. 52 are remarkably similar. In both cases the chromatic zones decrease in length at a much slower rate than do the achromatic zones. In both cases, the slopes of the lines do not go through the origin, indicating that there would be sizable pieces of chromatic material left even if the achromatic zones were to contract to zero. These residual chromatic lengths would be equal to 1.03 and 6.09 microns for the nucleolus-organizing chromosome and the translocation, respectively.

It is next of interest to consider the relative rates of chromosome contraction on the basis of the almost complete disappearance of the achromatic material as described above. If the achromatic material was converted to material indistinguishable from chromatic material, the chromosome as a whole would still be present after the achromatic material had apparently disappeared. If a straight line relationship were produced as a result of such a conversion, the straight line would not be expected to pass through the origin. If both chromatic and achromatic zones shortened at different rates but with no change of achromatic to chromatic material, the straight line would be expected to pass through the origin. The seemingly contradictory facts that the observed straight line does not go through the origin and that the achromatic material is not converted to chromatic material lead to a postulate of a very simple mechanism to account for them. This postulate will not only account for the observed relations but can be cytologically verified in part. The writer wishes to express his sincere thanks to Dr. Everett R. Dempster for suggesting this mechanism.

If the chromatic zones consist of alternate regions of heavy chromomeres resistant to contraction, and achromatic interchromomeric regions capable of contracting, then the chromatic zones would contract only until further contraction was blocked by contact of the chromomeres with one another (fig. 53). If the achromatic interchromomeric bands contracted at the same rate as the material of the achromatic zones, the relative rates of contraction of the chromatic and achromatic zones would give a straight line relationship. The slope of this straight line would be dependent upon the lengths of the chromatic and achromatic zones and the amount of interchromomeric material in the chromatic zones. Because of the blocking of the contraction of the chromatic zone by the heavy chromomeres, this straight line would not pass through the origin. That the chromatic zones do consist of chromomeres separated by rather lengthy achromatic interchromomeres may be seen clearly at zygotene in the tomato (fig. 4). Presumably these achromatic interchromomeric bands would continue to contract even after the chromomeres had come so close together that the interchromomeric regions were no longer clearly visible.

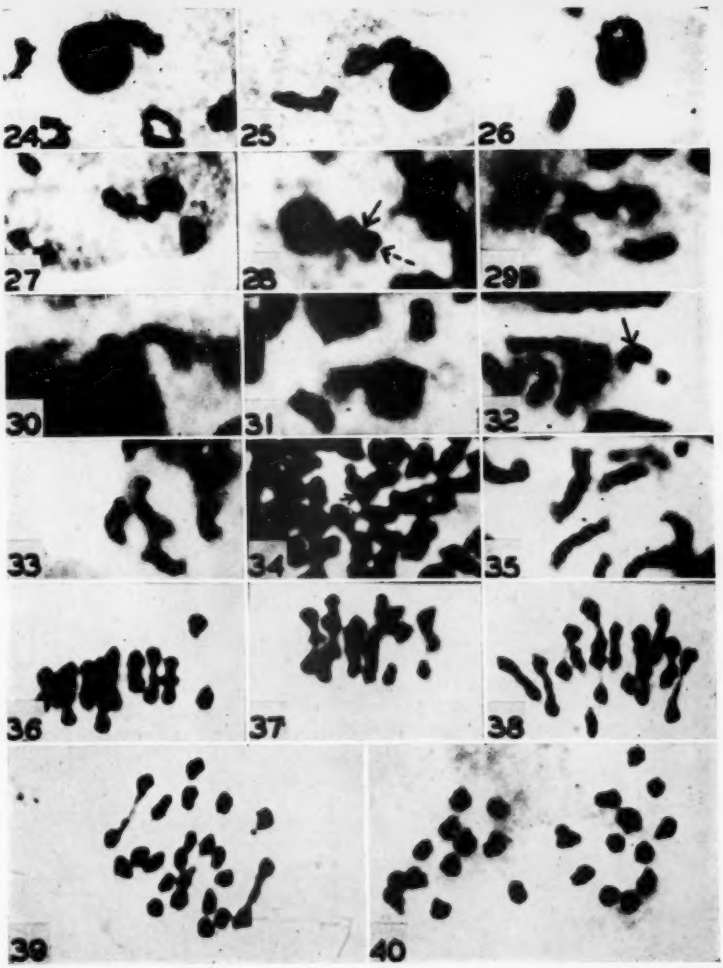

PLATE III

Early diakinesis to interphase, and mitotic stages. Figs. 28–33, 35, 3000×, others 2000×. Figs. 24–25. Nucleolus-organizing chromosome at middle diakinesis. Figs. 26–27. Nucleolus-organizing chromosome at late diakinesis. Figs. 28–29. Nucleolus-organizing chromosome at interphase. In fig. 28, straight arrow points to centromere constriction, broken arrow to longitudinal split. Figs. 30–31. Nucleolus-organizing chromosome with nucleoli at mitotic midprophase. Figs. 32–33. Nucleolus-organizing chromosome at late mitotic prophase. Arrow points to centromere constriction in fig. 32. Note the achromatic tail in fig. 33. Fig. 34. Nucleolus-organizing chromosome at mitotic metaphase. Arrow points to centromere constriction. Fig. 35. Chromosome at mitotic midprophase. Small achromatic region and telochromomeres are visible on the lower end. Fig. 36. Meiotic metaphase. Second and third bivalents from right have interstitial chiasmata. Fig. 37. Meiotic metaphase. Bivalent at left has large interstitial chiasma. Fig. 38. Early meiotic anaphase with achromatic zones between most of the separating bivalents. Interstitial chiasmata in the first and second bivalents from the left. Fig. 39. Early meiotic anaphase. Achromatic zones disappear as the bivalents complete their separation. Fig. 40. Late meiotic anaphase. Chromosomes show only chromatic material with exception of small achromatic ends of two chromosomes at the pole to the left.

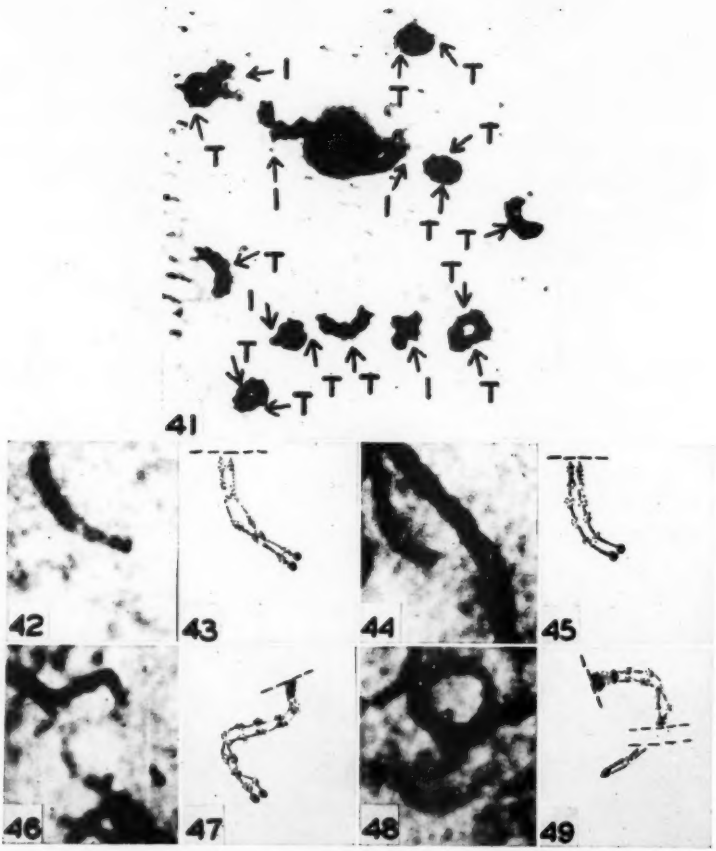

PLATE IV

FIGURE 41.—Diakinesis. 2000×. T, terminal chiasmata. I, interstitial chiasmata.

FIGURES 42–49.—Pachytene. 4000×. Figs. 42, 44, 46, 48. Photomicrographs showing delicate chromomere pattern in achromatic regions. Figs. 43, 45, 47, 49. Companion drawings, respectively, of the chromomere patterns.

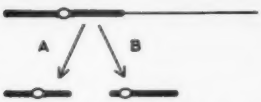

FIGURE 50.—Diagram illustrating the two possible results from different types of prophase behavior. If the achromatic material contracted to an extremely short length while the chromatic material contracted much less, a symmetric chromosome could result from an asymmetric one (arrow A). If the achromatic material became chromatized, the resultant chromosome would also be asymmetric (arrow B).

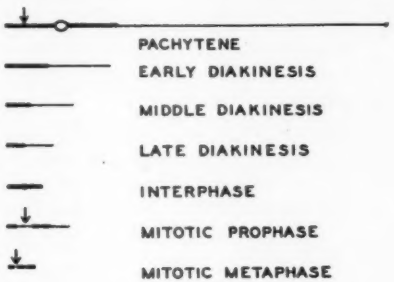

FIGURE 51.—Diagram based on measurements of lengths of various regions of the nucleolus-organizing chromosome during meiosis and mitosis. The vertical arrow at pachytene represents approximate region of attachment to the nucleolus. The broken line at mitotic stages (underneath arrows) represents the region where nucleolus is or has been. The centromere is represented as an oval in meiosis, and as a constriction in mitosis; it is not detectable during diakinesis.

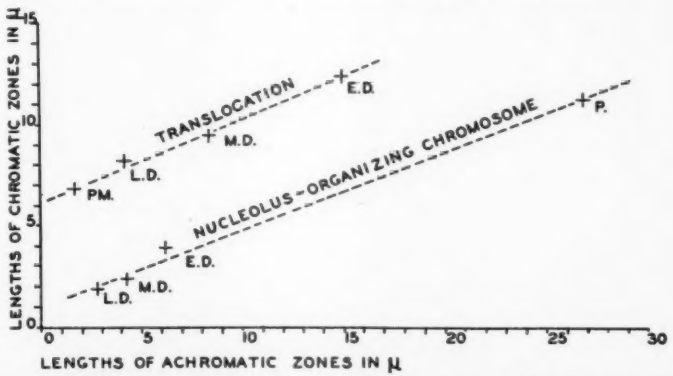

FIGURE 52.—Plots of the total lengths of the chromatic zones and the achromatic zones for the translocation ring-of-four chromosomes and the nucleolus-organizing chromosomes, P., pachytene; E.D., early diakinesis; M.D., middle diakinesis; L.D., late diakinesis, PM., prometaphase. The points represent averages for 15–25 measurements for the nucleolus-organizing chromosomes, and 25 measurements for the translocation rings. The straight lines were fitted to these averages by means of least squares; $y=6.09+0.41x$, for the translocation, and $y=1.03+0.37x$, for the nucleolus-organizing chromosome; y, length of chromatic zone; x, length of achromatic zone.

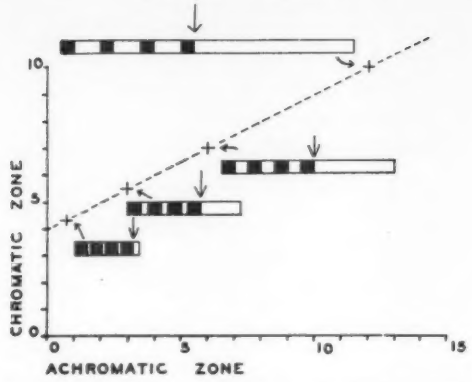

FIGURE 53.—Diagram illustrating an hypothesis of chromosome contraction. The horizontal bars represent chromosomes divided into chromatic zones, left of straight arrows, and achromatic zones, right of arrows. The chromatic zones are in turn divided into chromomeres, the black squares, and achromatic interchromomeric regions. The successively shorter bars represent chromosomes contracting. The achromatic, interchromomeric bands are assumed to contract at the same rate as the achromatic regions. The chromatic chromomeres are assumed not to contract. When the length of the entire chromatic zone is plotted against the length of the achromatic zone, a straight line which does not pass through the origin is obtained. No attempt has been made to illustrate coiling. Obviously, even a small amount of coiling would obscure the interchromomeric material in actual chromosomes.

DISCUSSION

The structure of the meiotic chromosomes of tomato resembles most closely those which have been regarded as differentiated into euchromatin and heterochromatin. The term, heterochromatin, was originally used by HEITZ (1929) to designate chromosomal material which maintained its metaphase stainability longer during telophase and acquired it earlier during prophase than the rest of the complement. In this report, the achromatic and chromatic zones of the tomato chromosomes have not been referred to as euchromatin and heterochromatin, respectively, for two reasons. The achromatic zones seem to shorten markedly without acquiring an appreciable capacity to stain darkly. Therefore they differ from the typical euchromatin, originally described by HEITZ, which does acquire during prophase a marked capacity for deep staining. Secondly, the various chromatic zones seem to show a differential behavior during the resting stage. While some apparently lose their stainability, others seem to clump together to form a chromocenter. Although much more study is needed to determine the exact process of chromocenter formation, it seems unwise to call all of the chromatic zones heterochromatin until their behavior in mitotic telophase nuclei can be further studied. In order to compare the behavior of the tomato chromosomes with that of other differentiated chromosomes, the achromatic and chromatic regions will be regarded as similar in some respects, if not identical to, eu- and heterochromatin.

Chromosome structure. At pachytene, the tomato chromosome may easily

be seen to consist of a centromere, proximal chromatic regions, distal achromatic zones, and telochromomeres. The morphology of the centromere is typical; its behavior in pairing will be considered later.

The chromatic zones are of interest in showing specific patterns of chromomeres. Chromomeres have been observed in heterochromatin in both meiotic and mitotic divisions. For the latter, DOBZHANSKY (1944) has pictured chromomeres of heteropycnotic regions in both the X and Y chromosomes of *Drosophila pallidipennis*. In plants, the meiotic chromosomes of Pellia (JACHIMSKY 1935) and Agapanthus and Kniphofia (DARLINGTON 1933) possess chromomeric heterochromatin. The B chromosome of maize is partially heterochromatic, and has a specific structure in the pycnotic regions (MCCLINTOCK 1933). It thus seems established that the heterochromatic regions may possess a chromomere pattern as specific as any found in euchromatic regions. In fact, in tomato the chromatic zones possess the obvious chromomere patterns, while those of the achromatic zones are much more difficult to distinguish.

DARLINGTON (1937) has discussed a type of heteropycnosis which appears near the centromere during meiosis, but not mitosis, in Agapanthus, *Zea mays* (the regular complement), Oenothera, and Canna. Because it appears in meiosis but not in mitosis, "It can, therefore, be represented simply as a timing difference at meiosis, the proximal parts being in advance of the distal parts. Moreover, since it applies to all chromosomes regularly in exactly corresponding regions it can scarcely be due to a differentiation of the genetic properties of the chromomeres; it must be due to the position of the precocious parts in relation to the centromere." (pp. 308–309, l. c.). Such is not the case in tomato where the chromomere patterns of the chromatic zones are variable from chromosome to chromosome among the complement, and constant for a particular chromosome. The possible effect of proximity to the centromere on the development of large amounts of stainable material can not be determined without experimental work.

Chromosome ends have frequently been associated with heterochromatin or heterochromatin-like structures or functions. Deep-staining chromosome ends have been observed at pachytene in Sphaerocarpus by LORBEER (1934). In *D. melanogaster*, tips of the salivary gland chromosomes frequently become fused with each other in the non-homologous fashion characteristic of the heterochromatin of these cells (PROKOFYEVA-BELGOVSKAYA 1937; HINTON and ATWOOD 1941). MULLER (1940) has postulated the existence of stable terminal ends of chromosomes, called "telogenes" or "telomeres." When a chromosome loses the telomere, it becomes labile and free to join with other such broken ends. Whether or not the telochromomeres have the role of making stable ends cannot be told by simple observational methods. Their seemingly typical, if not universal, presence in tomato indicates that they probably have some function, but this reasoning can not be extended to reveal what the function might be.

Chromosome pairing. In tomato, the centromeres may be observed to pair before the adjacent chromatic zones. Differential pairing behavior has been

reported for the centromere in Paris (HAGA 1944), and *Trillium kamtschaticum* (MATSUURA 1946). In these cases, some or all of the centromeres may remain in synaptic union until the first meiotic metaphase. In *Zea mays*, DARLINGTON (1934) noticed that the homologous centromeres are still paired until diplotene. In the tomato, the differential activity of the centromeres is apparent only because of the marked contrast with the pairing of the chromatic zones. Centromere pairing seems to be about synchronous with the pairing of the achromatic arms, and has completely disappeared by early diakinesis. Unfortunately the diplotene stage could not be studied to determine whether the centromeres separated more slowly than the other regions of the chromosome.

The slower pairing of the chromatic zones seems similar to pairing disturbances associated with heterochromatin. Different types of peculiar pairing behavior of the sex chromosomes of animals have been discovered in abundance (see WHITE 1945). In liverworts LORBEER (1934, 1936) observed a "distance" conjugation of the totally heteropycnotic sex chromosomes, and pairing in only the euchromatic regions of partially heteropycnotic chromosomes. On the contrary, MARQUARDT (1937) found that the heterochromatic regions of Oenothera paired very early in leptotene, while the euchromatin did not pair until zygotene. In Fritillaria and in Paris (DARLINGTON 1935, 1941, resp.) pairing was, in the first case, observed to begin in the proximal heterochromatic regions and proceed distally. In the second, it was believed to do so on the basis of localization of chiasmata.

Chiasmata. According to DARLINGTON (1937) tomato belongs to a class of organisms with completely terminalized chiasmata. UPCOTT (1935), and MACARTHUR and CHIASSON (1947) have both reported interstitial chiasmata at diplotene but claimed almost complete terminalization by diakinesis. On the other hand, AFIFY (1933) reported a terminalization coefficient of only 0.84 for diakinesis, and observed a retardation of bivalent separation at metaphase due to interstitial chiasmata. Although he was apparently unaware of the structural differentiation of the tomato chromosome, AFIFY's results are more nearly comparable to the present findings than are those of the other workers. The apparent reason for the difficulty in seeing the interstitial chiasmata of the tomato at diakinesis is that they seem to occur in the achromatic regions. Fixing and staining techniques which do not reveal the proper structure of the achromatic zones would lead one to believe that the bivalents (that is, chromatic zones) are held together by fine threads pulled out by terminalized chiasmata.

In other plants, chiasmata have been found in heterochromatic regions with a much lower frequency than in euchromatic regions, if at all. In Oenothera MARQUARDT (1937) found no chiasmata in the heterochromatic regions and JAPHA (1939) found only a few rare exceptions to this rule. In Agapanthus GEITLER (1933) found as many as four chiasmata per bivalent, all in the euchromatic regions. MATHER (1939) has concluded from statistical studies that interstitial chiasmata are formed where they are found, and that terminalization is an all-or-none process. If his idea is true also for differentiated chro-

mosomes, chiasmata are not produced, or produced only infrequently in heterochromatic zones, and their occurrence outside such zones can not be simply attributed to terminalization processes.

Chromosome contraction. JAPHA (1939) reported that the euchromatin of Oenothera contracted to 1/25 of its original length from pachytene to metaphase while the heterochromatic regions contracted to only 1/5. She did not disprove the possibility of a conversion of the euchromatin to a heterochromatin-like appearance. She believed that the differential rate was attributable to the fact that the heterochromatic material had already become strongly spiralized at pachytene. If contraction of chromatic regions of tomato were merely precocious, rather than blocked, the straight line relationships for their relative rates would pass through the origin, which they do not do.

Although spiral structure can occasionally be seen in the tomato chromosomes during mitosis, it is not necessary to assume that chromosome contraction in the tomato is attributable to spiralization, or to any other particular mechanism, to account for the hypothesis advanced in the preceding section. Spiralization has been found in such a wide variety of organisms (see review by SERRA 1947) that it is believed it will eventually be demonstrated in meiosis of tomato. Conventional techniques for revealing spiral structure have so far resulted in a complete disorganization of the chromosomes and an intense darkening of the cytoplasm.

By the technique of absorption of ultra-violet light, CASPERSSON (1938) found that the amount of nucleic acid in meiotic nuclei increased until leptotene but showed no further change as far as diplotene when the measurements were terminated. Because most of the chromosome contraction occurred after leptotene, CASPERSSON concluded that an increase in gross amount of nucleic acid was not necessary for contraction. He admitted that the nucleic acid might play a secondary role in chromosome contraction, but pointed out that if nucleic acid were responsible for chromosome contraction it should be present in the bands between the leptotene chromomeres. His study showed that nucleic acid was absent from the interchromomeric bands at leptotene just as it was absent from the material between the chromatic bands of the salivary gland chromosomes.

The material examined in the tomato is chromosomal substance differing in degree of stainability with aceto-carmine, and consequently the results of this study can not be compared directly with quantitative determinations of nucleic acid content. MIRSKY and RIS (1947a, b) have recently shown that a peripheral material which is responsible for chromosome staining may be removed leaving a residual strand which is much less chromatic. The peripheral material contained the bulk of the nucleoprotein of the chromosome.

Even if this suggestion of the greater nucleoprotein content of the tomato chromatic zones were to be discounted, there is still a remarkable similarity between the conclusions reached by CASPERSSON in his study of the amounts of nucleic acid and those reached here by a study of chromosome regions differing in amounts of stainable material. In both cases, an increase in amount of

nucleic acid, or of stainable material, does not seem to be necessary for chromosome contraction. Yet in both cases a very small amount may be all that is required, and this small amount may, after it is once acquired in early prophase, produce its effect through changes in its relationship with other components of the chromosome rather than by changes in amount.

SUMMARY

At pachytene, the chromosomes of tomato possess a highly differentiated structure. The centromere is surrounded on both sides by chromatic zones with specific chromomere patterns. The distal ends are achromatic and have a very delicate pattern of chromomeres which are difficult to detect. The achromatic regions form at least half the length of all chromosomes, and in some cases, much more than half. The ends of the chromosomes are typically terminated by distinct chromatic chromomeres, the telochromomeres.

The chromatic and achromatic zones are in some ways similar to the hetero- and euchromatin, respectively, of other organisms.

The chromatic zones pair at zygotene and early pachytene at a slower rate than do either the centromeres or the achromatic zones.

In the material examined, there was an average of 1.33 chiasmata per bivalent, and a terminalization coefficient at diakinesis of 0.70. In the long arm of the nucleolus-organizing chromosome, chiasmata were only 30 percent terminalized. In the completely chromatic short arm of the nucleolus-organizing chromosome, chiasmata occurred at a significantly lower frequency than would be expected from the pachytene length of this arm, if they occurred at all.

The achromatic zones contributed very little to the length of the contracted chromosome. This was demonstrated by following the reduction in length during meiotic prophase of an asymmetric chromosome with a long achromatic end.

The chromatic zones shorten at a rate much slower than that of the achromatic zones. The relative plots show a straight line relationship which does not pass through the origin. This may be most easily explained on the basis that the chromatic zones contain interchromomere regions which contract at the same rate as the distal achromatic zones while the chromatic chromomeres remain resistant to contraction.

ACKNOWLEDGMENTS

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THE PRODUCTION OF DOMINANT LETHALS WITH X-RAYS IN AGED DROSOPHILA MELANOGASTER SPERM

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IT is a well known fact that many different types of mutations can be produced by X-ray treatment. One of these types is called dominant lethals because an individual heterozygous for a mutant of this type dies before reaching maturity. Thus, it can be observed that there is a much higher death rate in the embryonic stage in the F_1 progeny from X-rayed *Drosophila melanogaster* flies as compared with the progeny from non-irradiated parents. Less striking is the corresponding difference in death rate in the larval and pupal stages. The frequency at which dominant lethals occur in these different developmental stages when the parents have been treated with a certain amount of X-rays is described by several investigators (HANSON 1928; TIMOFÉEFF-RESSOVSKY 1937; DEMEREC and FANO 1944). The cytological aspect of the problem is described by SONNENBLICK (1938, 1940). The more theoretical and mathematical parts of the problem are treated by PONTECORVO (1942) and LEA and CATCHESIDE (1945). However, the question as to whether the age of the gametes at the time of X-ray treatment alters the percentages of dominant lethals induced has received relatively little attention. DEMPSTER (1941) reports that he noticed an increase in dominant lethals induced by X-rays correlated with an increase in age of the sperm at the time of irradiation. The author has taken up this problem for investigation and has obtained results in agreement with those of DEMPSTER.

MATERIAL AND EXPERIMENTAL METHODS

Stocks of *Drosophila melanogaster* were obtained from the Division of Genetics, UNIVERSITY OF CALIFORNIA. Males of an Oregon R-C stock (our No. S-6) were irradiated. The stock had been maintained by brother-sister matings with occasional half brother-sister matings for the last nine years.

In order to obtain egg laying females possessing hybrid vigor and capacity for producing eggs with high hatchability (when mated with unirradiated males), females of a strain homozygous for the genes *cn*, *bw*, and e^{11} were crossed with wild type males of the Canton S stock. The F_1 virgin females were used as egg layers in all the experiments and were mated either with irradiated or control males of the inbred Oregon R-C stock. The eggs from these matings were counted to determine the percentage of hatchability.

All male flies were etherized three times: once when collected to determine sex, secondly when placed in the gelatin capsule, and thirdly when placed in the creamer jar. Females were etherized twice—during the first and third of the above processes.

The cultures were raised in half-pint bottles containing the usual cornmeal-molasses medium with moldex added to prevent moldiness, and one percent dried brewers yeast (autoclaved before being added). A drop of Fleischmann's yeast suspension was added to each bottle. All cultures were raised at a temperature of 26° ± 1°C.

In collecting males for irradiation, a number of stock bottles were made up, and this stock was supplied with new bottles every day so that the number of flies was always large enough to furnish a sufficient number of males. The males were collected every day from these bottles and stored until the time of irradiation. In this way flies with an age difference of one day were collected. Males from 0 to 24 hours old were labeled 0 days old; those from 24 to 48 hours were labeled 1 day old, and so on.

In order to collect eggs, rectangles (12×17 mm) of black filter paper dipped in a culture medium of mashed banana and a four percent agar solution were made up (POWSNER 1935). A coat of very dilute yeast suspension was added. Each rectangle was then placed on a cardboard base, and these were stored by placing them on wet paper towels in a closed container until they were used.

When ready for collecting eggs, a small creamer (that is, a miniature milk bottle of 20 cc capacity) was used to cover the filter-paper-rectangle and a single pair of flies was placed in each creamer. Each creamer jar and each plaque was so marked that there was no possibility for inter-change or other mistakes. The eggs laid during the first 24 hours were discarded because some might be laid before mating takes place and also eggs are laid so rapidly immediately subsequent to mating that not all of them may be fertilized. Eggs for counting were laid during 6–8 hour periods. A count was then made of the eggs on the plaques, and the latter were then placed on wet paper towels in a box at a temperature of 26° ± 1°C. After 24 hours the eggs were again counted to determine the percentage of hatchability (POWSNER 1935). A Spencer dissectoscope (×25) was used to count the eggs. Hatched eggs were easily recognized by their flattened and wrinkled appearance.

TYPES OF RADIATION AND MEASUREMENTS

A General Electric Maximar 220 kilovolt X-ray apparatus, made available through the courtesy of the WILLIAM H. CROCKER RADIATION LABORATORY, was used for all irradiations. MR. HAL ANGER of the DIVISION OF MEDICAL PHYSICS was kind enough to perform the first irradiation test. The subsequent irradiations were made jointly by DR. EVERETT R. DEMPSTER and the author.

Many small doses were used so that each dose could be measured by a 250 r-unit thimble chamber of a Victoreen dosimeter. The tube was operated at 200 kilovolts 10 milliamperes with a 1 mm aluminum and a 0.25 mm copper filter.

Immediately preceding the administration of radiation, flies were placed in a No. 5 (5×11 mm) gelatin capsule which had been pierced at each end.

In the first experiment the flies were placed at a distance of 41 cm from the target. In all the remaining experiments they were placed at a distance of 31 cm from the target.

GENERAL CONSIDERATIONS REGARDING THE RAW DATA AND THEIR STATISTICAL TREATMENT

A tendency of hatchability to decrease with increase in age of irradiated sperm is apparent from inspection of the raw data. In order to determine whether this tendency is significant or whether the variation between groups at different ages might be due to chance, a chi square test was made. Although the chi square between ages in the irradiated groups was always highly significant, the chi square within ages was also greater than could be accounted for by chance alone. This test, therefore, is not conclusive. Analysis of variance indicates significantly greater variation between than within groups, but this test is open to the criticism that the individual tests would not have the same variance. A test of KENDALL's (1938) rank correlation coefficient between

TABLE 1

Experiment I. The effect of the age of Oregon R-C sperm when not irradiated, and when irradiated with a dosage of X-rays 2219 r

AGE IN DAYS	MEAN PERCENTAGE HATCHABILITY	
	UNIRRADIATED FLIES	IRRADIATED FLIES
1	96.83	61.27
2	95.99	51.97
3	98.06	—
4	96.43	50.23
5	92.76	—
6	97.85	—
7	91.83	—
8	98.87	50.33
9	100.00	—
10	98.95	—
11	97.86	—
12	97.32	—
13	94.48	—
14	94.53	—

percentage hatch and age of sperm at time of irradiation is a conservative one in that there is no assumption as to form of distribution or variance of individual tests. The only assumption is that tests at different ages are independent. A statistically significant rank correlation therefore establishes with a high degree of certainty that hatchability does depend on age of sperm at time of irradiation. The results of the statistical tests will be discussed in connection with the individual experiments.

EXPERIMENT I

The Oregon R-C males were given an X-ray treatment with 2219 r and mated to virgin females heterozygous for *cn*, *bw*, and e^{11}. Due to an accident in the etherizer, only a few classes recovered from the ether. The treated males

TABLE 2

Analysis of the data obtained in experiment I

	EGGS FERTILIZED BY IRRADIATED SPERMS	EGGS FERTILIZED BY UNIRRADIATED SPERMS
Rank correlation between hatchability and age groups	.67	−.011
Level of significance	.17	.96

of age groups 1, 2, 4, and 8 days old were maintained throughout the experiment. Untreated males from the same stock were used as controls. Due to the loss of many of the irradiated males, this experiment was carried through primarily to obtain a reliable value for hatchability in the control. In this experiment 5588 eggs were counted. Table 1 presents in a condensed form the data obtained in this experiment. The statistical treatment of these data is presented in table 2. In the control series the rank correlation does not approach statistical significance. In the irradiated series, on the other hand, the rank correlation, although not significant, is in the direction anticipated and

TABLE 3

Experiment II. The effect of age of Oregon R-C sperm when not irradiated, and when irradiated with a dosage of X-rays 2301 r

AGE IN DAYS	MEAN PERCENTAGE HATCHABILITY	
	UNIRRADIATED FLIES	IRRADIATED FLIES
0	96.70	55.18
1	—	55.71
2	—	57.11
3	95.36	51.79
4	—	43.41
5	—	50.00
6	94.37	48.76
7	—	35.52
8	—	44.79
9	93.06	46.68
10	—	44.66
11	—	47.88
12	99.05	43.27
13	—	41.50
14	—	43.11
15	97.92	43.99
16	—	32.62
17	—	38.07
22	95.77	38.71

fairly high in magnitude. A larger sample is required in order to establish that the correlation obtained is not due to chance.

EXPERIMENT II

This experiment was conducted in the same way as the first experiment. Males were exposed to 2301 r units of X-rays. Flies of the following age were used: Eighteen classes from 1 to 17 days, inclusive, and one class of 22 days. Untreated males of the same stock were used as a control. The experiment was performed with six single pair matings in each class in the treated series and three single pair matings in each class in the control. In this experiment 14,369 eggs were counted.

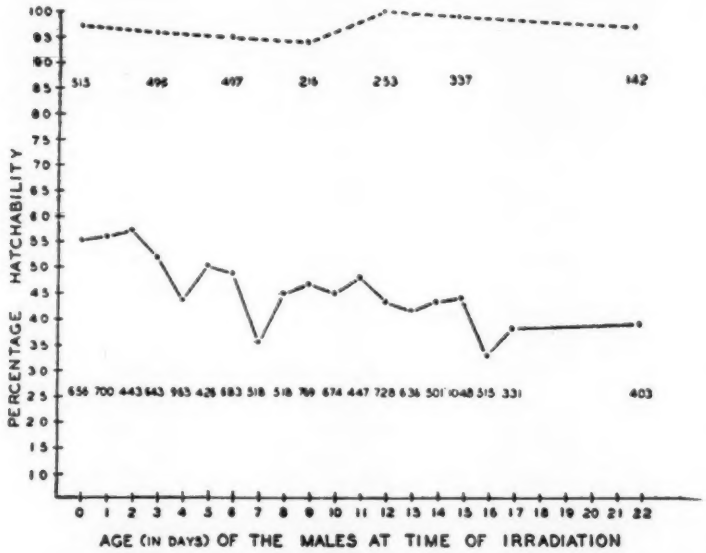

FIGURE 1.—Hatchability of eggs fertilized by irradiated sperms related to age of males at time of irradiation. The numbers above a given abscissa represents the numbers of eggs in the corresponding age group from which the curve above is computed.

——— indicates males from Oregon R-C given a dose of 2301 r.

——— indicates males from Oregon R-C not irradiated.

Table 3 and figure 1 present in condensed form the data obtained in experiment II, and table 4 summarizes the results and analyses.

Here, as in the first experiment, the rank correlation is not at all statistically significant for the controls but, however, so highly significant for the irradiated series that it may be safely concluded that the tendency for hatchability to decrease with age of the sperm at the time of irradiation cannot be attributed to chance.

TABLE 4

Analysis of the data obtained in experiment II

	EGGS FERTILIZED BY IRRADIATED SPERMS	EGGS FERTILIZED BY UNIRRADIATED SPERMS
Rank correlation between hatchability and age groups	.59	.05
Level of significance	.0004	.88

TABLE 5

Experiment III. The effect of the age of Oregon R-C sperm irradiated with 2306 r in males stored alone and in males stored with an excessive number of females

AGE IN DAYS	MEAN PERCENTAGE HATCHABILITY	
	MALES STORED ALONE	MALES STORED WITH FEMALES
1	54.44	48.91
3	50.14	46.38
5	47.39	56.43
7	47.73	49.42
9	41.09	54.90
11	50.45	41.33
13	34.45	45.07
15	43.24	50.70
17	40.24	32.01
19	41.73	13.51
21	44.99	34.15
23	43.80	0.00

EXPERIMENT III

Males of Oregon R-C stock were collected every day. These were divided into two lots. One lot was stored as in previous experiments. The other lot was placed in bottles with an excessive number of females. In this case all of the males were exposed to a dosage of 2306 r units of X-rays. The flies differed in age by two days in a series from 1 to 23 days old. A total of 5536 eggs were counted in this experiment, and the data obtained are presented in a condensed form in figure 2 and in table 5. This table lists the mean percentages of hatchability for different age groups of eggs fertilized by irradiated males that had been previously stored with an excess of females. It is most likely that the males in this group have not been storing their sperms and, hence, males of different age will all have sperms of the same age. Therefore, eggs fertilized by sperms from irradiated males in this series will be expected to give nearly the same percentage of hatchability. Inspection of these data reveals that there

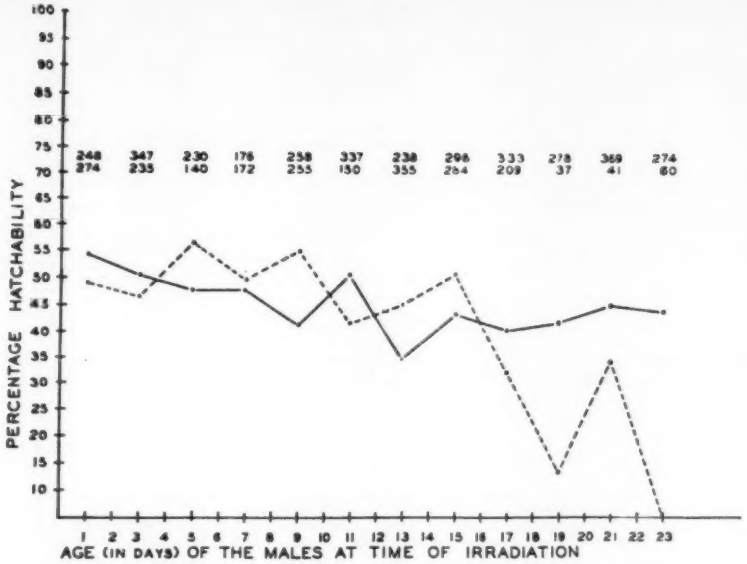

FIGURE 2.—Hatchability of eggs fertilized by irradiated sperms related to age of males at time of irradiation. All males were from Oregon R-C and were given a dose of 2306r.

——— indicates males stored alone. The first line of numbers above the abscissa represents the number of eggs in each age group from which this curve is computed.

------ indicates males stored with an excess of females. This curve is computed from the second line of numbers above the abscissa.

was only a little change in hatchability with age up to the fifteenth day but that following this period there was a pronounced drop. It is possible that this sudden drop is due to exhaustion of sperms rather than of a greater sensitivity of older flies to the irradiation. For this reason two analyses were made of the data obtained—the first including all the data, and the second excluding eggs fertilized by sperms from males over fifteen days of age when irradiated. Tables 6 and 7 summarize the results and the two sets of analyses of this experiment. The result of these two analyses involving the eggs fertilized by males

TABLE 6

Values for analyses of all data obtained in experiment III

	EGGS FERTILIZED BY IRRADIATED SPERMS FROM ♂♂ STORED ALONE	EGGS FERTILIZED BY IRRADIATED SPERMS FROM ♂♂ STORED WITH ♀♀
Rank correlation between hatchability and age groups	.36	.45
Level of significance	.10	.05

TABLE 7

Values of analyses, excluding eggs fertilized by sperms over 15 days of age (experiment III)

	EGGS FERTILIZED BY IRRADIATED SPERMS FROM ♂♂ STORED ALONE	EGGS FERTILIZED BY IRRADIATED SPERMS FROM ♂♂ STORED WITH ♀♀
Rank correlation between hatchability and age groups	.36	.07
Level of significance	.10	.80

stored with females indicates that whatever significance there is in rank correlation is due almost entirely to the last three age groups (the 23 day age group was not included in either analysis). The results for aged sperms are in agreement with the previous experiments, although in this sample the rank correlation did not reach statistical significance.

EXPERIMENT IV

This experiment was carried out chiefly to determine whether early or late development would make any difference in sensitivity to exposure to X-rays. Parent flies from an Oregon R-C stock were allowed to deposit eggs for ten hours and then all the parent flies were removed. When the first fly started emerging from this culture, all those emerging through the first day were collected. Flies emerging on the second, third, and fourth day were discarded, and males emerging on the fifth day were collected. Thus males with a difference of four days in their time of development were obtained. Each of these batches was divided into two groups. One group of males from each batch was irradiated when the males were one day old. The other group of males was irradiated when the males were 15 days old. The experiment was otherwise carried out in the same fashion as all previous experiments. A total of 1703 eggs were counted.

Early emerging males, one day old and exposed to 2304 r of X-rays, gave a mean percent of hatchability $=51.25$. Late emerging males, one day old and exposed to 2296 r of X-rays, gave a mean percent of hatchability $=31.97$.

An analysis of variance was made of eggs fertilized by individual males within and between groups emerging on the first and fifth day for those one-day-old flies and gave a P value much less than .01. This P value clearly indicates that samples of early and late developing males had sperm of different sensitivity to induction of dominant lethal mutations by X-rays.

Early emerging males, 15 days old and exposed to 2308 r of X-rays, gave a mean percent of hatchability $=35.21$.

Late emerging males, 15 days old and exposed to 2206 r of X-rays, gave a mean percent of hatchability $=34.86$.

An analysis of variance was made again and a P value much higher than .05 was obtained. This indicates that the difference between groups is not significant compared with the variation within groups.

A test of all the data in this experiment yielded a within groups chi square of 18.61 with 20 degrees of freedom ($P > .8$). This indicates that most of the difference in hatchability within age groups must be due primarily to early and late development of the larvae.

Later experiments by the author show that eggs which were fertilized by sperms from early emerging males and which were collected on consecutive days exhibit only a moderate variation in hatchability. Those eggs which were fertilized by late emerging males showed a rapidly decreasing hatchability. Further experiments are in progress to investigate this part of the problem.

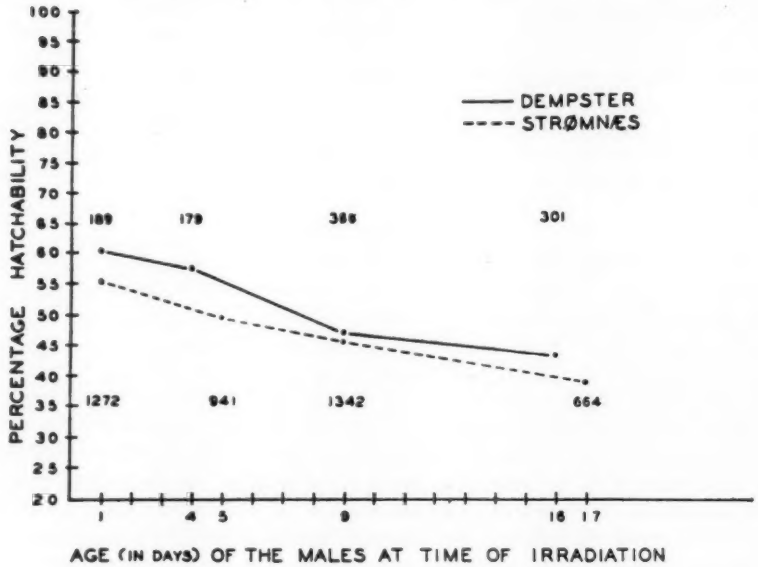

FIGURE 3—Comparison of results found by DEMPSTER and STRØMNÆS on the hatchability of eggs. These eggs were fertilized by sperms given a dosage of about 2300 r of X-rays. The numbers above a given abscissa represent the numbers of eggs from which the curves are computed.

DISCUSSION

In these experiments 213 males have been treated with X-rays and a total of 23,196 eggs have been counted. The detailed data are too numerous to present in this paper and, therefore, only the mean percentage of hatchability for each single age group is given. No selection of data was carried out except that completely sterile cultures were excluded. Several investigators have found that the percentage of dominant lethals increased approximately linearly with dosage up to 5000 r units and that a mutation rate of one percent would be produced by 40–50 r units. Therefore, by using 2300 r units, the expected mutation rate should be around 50 percent. It was possible to apply nearly identically the same dosage in each experiment. The largest deviation in ex-

periments II to IV is 10 r units and should, therefore, account for a maximum variation of less than 0.25 percent of the lethal mutations actually observed. Therefore, no correction is necessary in order to compare the results in the various experiments.

POWSNER (1935) shows that the duration of egg period (that is, the time from which the egg is laid until it hatches) is about 21 hours at a temperature of 25°C for eggs laid by *Drosophila melanogaster* females. In the experiment described in this paper, the eggs determining the percentage of hatchability were counted and then stored at a temperature of $26° \pm 1°$C. To make sure that eggs were not hatched after 24 hours, several plaques with eggs fertilized by sperms from both irradiated and unirradiated males were given an additional 24 hours storage at the same temperature. The percentage of hatchability was determined again and found to be the same after 48 hours as after 24 hours of storage. Furthermore, the differences between hatched and unhatched eggs are so distinct that there should be no reason for mistake.

Flies begin egg laying when they are two days old and lay very few eggs as long as they are young virgins, but after copulation the egg production increases considerably (PATTERSON, WELDON BREWSTER and WINCHESTER 1932). The hatchability is expressed as the percentage of the total number of eggs laid that hatched. Some eggs fail to hatch because they are not fertilized or because they are killed by dominant lethals. To assure that only fertilized eggs were counted, the first batch was always discarded.

KAUFMANN and DEMEREC (1942) found that the percentage of hatching dropped from around 90 percent to 0 percent in ten consecutive days of egg laying after the female had copulated only once. The highest degree of fertility was found on the second day of laying. They performed the same experiment with the males previously irradiated with 3000 r units of X-rays and found that the percentage of fertile eggs dropped from 25 percent to 0 percent over a period of 14 days of egg laying. These experiments show that the females receive enough sperms in one copulation to fertilize the eggs deposited on a few consecutive days only. In the light of these considerations, the males were allowed to stay with the females as long as the experiment was running. Thereby an ample supply of sperms to fertilize all the eggs laid should have been secured. There were some occasions where males either escaped or died, and in such instances the mate was allowed to lay eggs on two consecutive days and was subsequently discarded. According to HUETTNER (1930) and DEMEREC and KAUFMANN (1941), it was found that males which exhausted all their available sperm in one day may have functional sperm on the succeeding day. In the case of irradiated males, these sperms show no significant difference in the proportion of dominant lethals. DEMEREC and KAUFMANN observed an appreciable drop in the percentage of sex-linked lethals occurring 15 to 19 days after irradiation for sexually active males, and for dominant lethals a corresponding drop was not observed until the nineteenth day. These drops in hatchability have been attributed to the maturation of sperms which were immature at the time of treatment. Therefore, eggs were never collected for a period longer than ten succeeding days in order to secure a count of eggs fertilized only by sperms which were mature at time of irradiation.

DUNCAN (1930) tested the fecundity of *Drosophila melanogaster* males. He found that the average fertile period was about 32 days. Sperms were observed in the testes of all males after their fertile period was over. No sperms, however, were found in the sperm receptacles of females that had been mated to these males during their sterile period. DUNCAN concluded, therefore, that impotency of the old males must be due to a loss of function in the ejaculatory apparatus. Unfortunately DUNCAN does not give any information about the relative fertility of the individual males. It seems improbable that the fertility should drop from close to 100 percent down to 0 percent in a 24 hour period. A continuous decrease in the relative fertility seems to be more likely. The sharp drop observed in experiment III, where males were stored with an excess of females, might be explained on the basis of DUNCAN'S investigation. The author made a test where males were stored with an excessive number of females (1 male: 5 females) for a period of 1, 3, 5 days, and so on up to 25 days. These unirradiated males were then mated to virgin hybrid females as described earlier. The results indicate that a drastic drop, as observed in experiment III, does not occur until the 25 day male group is reached. Therefore, one might find it reasonable to conclude that the infertility observed in experiment III of males older than 15 days is partially due to the previous high level of sexual activity of the males and partially due to the radiation of very young and immature sperms. DEMEREC and KAUFMANN (1941) describe how determinations can be made, from the counts on eggs laid, eggs hatched, and pupae and flies emerging therefrom, of death rates during the egg, larval, and pupal stages. The determinations indicate that the alterations induced in the sperm by 3000 r treatment kill the great majority of individuals in the embryonic stages and only a few in the larval stages. They found no evidence of excessive death rate among the pupae as compared with the controls. As the dominant lethals express themselves mostly in the embryonic stages and to a lesser degree in the larval stages, the dominant lethals induced in the sperms by the X-ray treatment have been determined in the embryonic stage throughout this investigation. The conclusion that unhatched eggs are killed by dominant lethals is based on the assumption that eggs laid by an impregnated female have been fertilized. This assumption would not be true if copulation should stimulate a female to lay unfertilized eggs. Since polyspermy occurs in *Drosophila melanogaster*, one would expect that fertilization normally takes place. To settle this question DEMEREC and KAUFMANN studied 99 eggs laid by females and fertilized by sperms previously X-rayed with 5000 r units, and they found that only one of the 99 eggs was not fertilized. It seems, therefore, a justifiable assumption that the unhatched eggs were killed by dominant lethals.

SONNENBLICK (1940) made cytological observations of eggs fertilized by X-rayed sperms. He found fragmentation, chromosome clumping and multipolar configuration in the cleavage figures.

CATCHESIDE and LEA (1945) compare data from SONNENBLICK and from their own experiments of induction of dominant lethals. They find it rather unsatisfactory that there exists a real quantitative difference in their curves

for the mutation-dose relation. The difference is larger than can be due to errors in dosimetry in modern experiments, and it cannot be ascribed to the organism used either, since both have used Oregon-R males. In accordance with the hypothesis put forth in this paper, this discrepancy, however, may easily be explained if one supposes that they have been irradiating sperms of different age.

PONTECORVO (1942) studied the problem of dominant lethals. He came to the conclusion that most dominant lethality would be determined by single-break sister unions at low dosages and that as the dosages increased, other lethal changes (such as translocations and deletions) would come to play an increasing part. Hence, the approximately "one hit" type is correlated with low dosages and the "two or more hit" type is correlated with higher dosages. DEMEREC and FANO (1944) came to the same interpretation of the origin of dominant lethals. However, they found that the dependence of the sex ratio on the dosage required some modification of this interpretation. A quantitative theory of dominant lethals is developed by LEA and CATCHESIDE (1945) on the basis that dominant lethals are a mixture of single breaks which fail either to restitute or to interchange but instead undergo sister-union, and lethal chromosomal structural changes involving two or more breaks. The experimental results cited by them fitted this theory even where it concerned the distortion of the sex ratio in the progeny of irradiated males with ring-shaped or rod-shaped X-chromosomes.

PATTERSON, WELDON BREWSTER, and WINCHESTER (1932) found that virgin females store up a comparatively large number of eggs in their ovaries and deposit very few while mated females do just the reverse. The virgin females were aged 1, 3, 5, 7 and 9 days at time of irradiation. They were then given a dose of 795 r units of X-rays and the number of fertile eggs was determined. The conclusion arrived at was that X-radiation increases the percentage of infertility, of primary non-disjunction, and of breakage of the X-chromosome, when applied to mature eggs that have been "aged" by retention in the ovaries. DEMPSTER (1941) investigated the hatchability of eggs fertilized by sperms from 18 males stored alone in 4 age groups where the males had been treated with 2300 r units of X-rays. He found that the survival of eggs fertilized by sperms irradiated when they were one day old was 60.32 percent, and when they were 4 days old, 57.54 percent, when 9 days old, 47.01 percent, and when 16 days old, 43.19 percent. The author's own observations from three different experiments, correlating very nearly with the ages of those above, gave the following percentages: For 1-day-old sperms, 55.35 percent, for 5-day-old sperms, 49.73 percent, for 9-day-old sperms, 45.69 percent, and for 17-day-old sperms, 39.16 percent. These are somewhat lower percentages than those observed by DEMPSTER, but otherwise the tendency for an increased sensitivity to X-ray irradiation correlated with age is present in both cases (figure 3).

SUMMARY

By storing males alone without females it is possible to age the sperms. When such sperms of different age groups have been irradiated, it has been

found that the sensitivity to X-rays increases with increase in age. This is in agreement with DEMPSTER (1941). The age effect is also found when eggs have been aged (PATTERSON, WELDON BREWSTER, and WINCHESTER 1932). No age effect is found when the males have been stored with an excess of females. The age effect, however, seems to be found only in sperms from early developing males. The dependence of mutation frequency on age of sperm and rate of development of the males irradiated may explain the discrepancies between the frequency dosage relationships for dominant lethals reported by different authors.

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